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THE TIME FACTOR IN THE IRRADIATION OF MALIGNANT TUMORS

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Experimental studies of the effect of divided doses of radiation on tumors are almost an untouched field. The only important studies of this type reported in the literature are those by Juul,[1] who irradiated tumors of mice with roentgen rays and radium. His findings were as follows: A given dose was more effective when applied in a single exposure than when divided. However, with the single dose there was an undesirable skin reaction, and since the cumulation of the skin and that of the tumor were different, there was a more favorable ratio between the two with fractioning. Juul also irradiated tumors of mice by the massive, the fractional and the saturation method, respectively, in such a manner that the effect on the skin was the same with all three methods. "The effect on the tumors proved feeblest in the case of those that had been irradiated with one massive dose; somewhat stronger from the simply divided irradiation; and considerably stronger, again, from the dosage by Pfahler's method." Eidinow and Mottram [2] and Ludford [3] investigated a few tumors with divided doses and found no difference in effect between the divided dose and the single exposure.

Our experiments are similar to those of Juul. In addition, we have compared a fourth method with the massive, the fractional and the saturation method, namely, a modified form of the protracted fractional treatment. Further, and more important, we have studied the histologic

Aided by a grant from the Cancer Research Institute of the Chicago Woman's Club.

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1. Juul, J.: Acta radiol., supp. 9, 1929, p. 1; **11**:102 and 226, 1930; Strahlentherapie **38**:623, 1930.

2. Eidinow, A., and Mottram, J. C.: Lancet **1**:1236, 1931.

3. Ludford, R. J.: Scient. Rep. Invest. Imp. Cancer Research Fund **10**:125, 1932.

changes in tumors irradiated by the divided dose methods—a type of study which has not previously been made.

EXPERIMENTAL PROCEDURE

The objective of the experiment was to keep all factors constant except the time factor. The latter was to be variable. Rat tumors, all exactly the same age and about the same size, growing in 155 animals, all about the same age and weight, were irradiated with roentgen rays of constant intensity and quality.

Three different transplantable tumors were studied. They were procured from Dr. Francis Carter Wood. They were the Flexner-Jobling rat carcinoma (strain 223-I), the Jensen rat sarcoma (strain 342-A) and the Walker rat tumor 256 (strain 133-B), a carcinosarcoma. An ordinary laboratory strain of white rats was used. Immature males and females about 5 to 6 weeks old and of fairly uniform size were kept eight to ten in a cage; as the study advanced, they were subdivided into smaller groups, and finally each animal was placed in an individual cage. They were given all they could eat of a standard commercial ration containing all food factors and were given also plenty of water. They were identified by painting parts of the fur with various dyes. Very small pieces of tumor were inoculated under sterile conditions, with a large bore aspirating needle and plunger. The tissue was inoculated subcutaneously in the middle region of the back. Within from five to seven days tumors were palpable as small nodules at the site of implantation, and within ten days they were about pea size.

The Flexner-Jobling carcinoma was inoculated into sixty-six rats, with fifty-eight takes; forty-four of the tumors were used in the studies of treatment, eleven were used for additional histologic studies, and three underwent spontaneous involution. The Jensen sarcoma was inoculated into seventy-three rats, with sixty-three takes; forty-four were used in the studies of treatment, seventeen were used for additional histologic studies, and two underwent spontaneous involution. The Walker tumor was inoculated into only sixteen rats, with ten takes. This formed too small a series to be of significance, although the results of treatment in this group agreed with those in the other two groups.

The treatments were all started on the tenth day after inoculation. The tumors were measured every day during the first two weeks, and after that, twice a week. Six weeks after the beginning of treatment all the rats that remained were killed, and the tumors that were left, as well as skin and other organs, were examined histologically. During the course of the treatments several rats were killed, and others died from various causes. Tumors, skin and in representative cases other tissues from these animals were examined histologically. All the tissues were fixed in 10 per cent formaldehyde and embedded in paraffin. Sections were stained by the hematoxylin eosin method, the Van Gieson method for collagen, the Weigert method for elastic tissue and the Kossa method for identification of calcium.

The factors in the roentgen treatment were as follows: a radiation peak of 120 kilovolts, obtained by a Standard apparatus with mechanical full wave rectification; a current of 5 milliamperes; an aluminum filter 0.5 mm. thick, and a skin focus distance of 8 inches (20 cm.). These factors were selected as combining sufficient penetrating power for the application of identical doses to small tumors just beneath the skin and sufficient intensity for the application of an erythema dose in a short time. The half value layer of this radiation in water was from 1.4 to 1.5 cm. The intensity of the rays was 185 roentgens (r) per minute. The roentgen rays were applied through an opening in a piece of lead rubber with allowance of only a small margin outside the tumor. The rats were kept under

light ether anesthesia during the first two weeks of the experiment; later they were tied to a small board.

The animals were divided into five groups as follows:

1. Controls, not exposed to the rays.

2. Rats given massive treatments, i. e., from one to sixteen minutes' irradiation (185 to 2,960 r) at one time.

3. Rats given fractional treatments, i. e., one-fourth, one-half or one minute's irradiation (46¼, 92½ or 185 r) daily from four to thirty times, the total dosage ranging from 370 to 5,550 r.

4. Rats given subfractional treatments, i. e., a modified form of the protracted fractional method. Fractional treatments exactly the same as those in group 3 were subdivided into shorter treatments of from five to ten seconds each, with two minute intervals, so that the total time of treatment was considerably prolonged (up to twelve minutes) without change in the intensity or quality of the rays. In this way, no variations in quality could occur due to variation in thickness of filter, skin focus distance or kilovolt-time curve depending on milliamperes.

5. Rats given saturation treatments. From one to six minutes' irradiation (185 to 1,110 r) was given as an initial treatment, followed by daily application of one-fourth or one-half minute (46¼ or 92½ r).

FLEXNER-JOBLING CARCINOMA

1. *Untreated Controls (Fig. 1 A).*

Gross Observations.—Seven rats with tumors of this type were left untreated as controls. Two of the tumors were fixed in formaldehyde solution at ten days for histologic examination. The remaining five increased in size until they caused death by invasion or metastasis or until the rats were killed, at the end of the experiment.

Histologic Observations.—The Flexner-Jobling carcinoma is an undifferentiated squamous cell carcinoma (fig. 2 *A*). In the controls, at ten days, the tumor was composed of irregular cords of large polygonal cells. The cell outlines were fairly distinct, and the cytoplasm was clear and eosinophilic. The nuclei were large and varied in shape from round to slightly oval; each was vesicular, with a clearly defined nuclear membrane, a loose chromatin net composed of fine and coarse granules of chromatin, and from one to three prominent eosinophilic nucleoli. Occasional mitotic figures were seen (about 1 per high power field), which were fairly regular, although a few were slightly abnormal. An occasional nucleus was larger than the rest, although no giant forms were seen. Between the cords of tumor cells there was a moderate amount of connective tissue, which was fairly cellular and in which an occasional blood capillary was visible. The tumor was oval and rather regular in outline, and about it was a narrow zone of cellular connective tissue infiltrated by lymphocytes. As it grew there was no change in its histologic structure. At four weeks it was larger; the center was composed uniformly of necrotic eosinophilic granular material infiltrated by polymorphonuclear leukocytes, but the periphery was composed of intact tumor of exactly the same character as the younger tumor. At six weeks the tumor was exactly as it was when observed at four weeks, except that it was larger and that occasional moderate-sized mononuclear giant cells were seen (about 1 per four high power fields). In the zone at the junction of intact and necrotic tumor, the cells could be seen in various stages of spontaneous necrosis, with pyknosis of the nuclei and karyorrhexis.

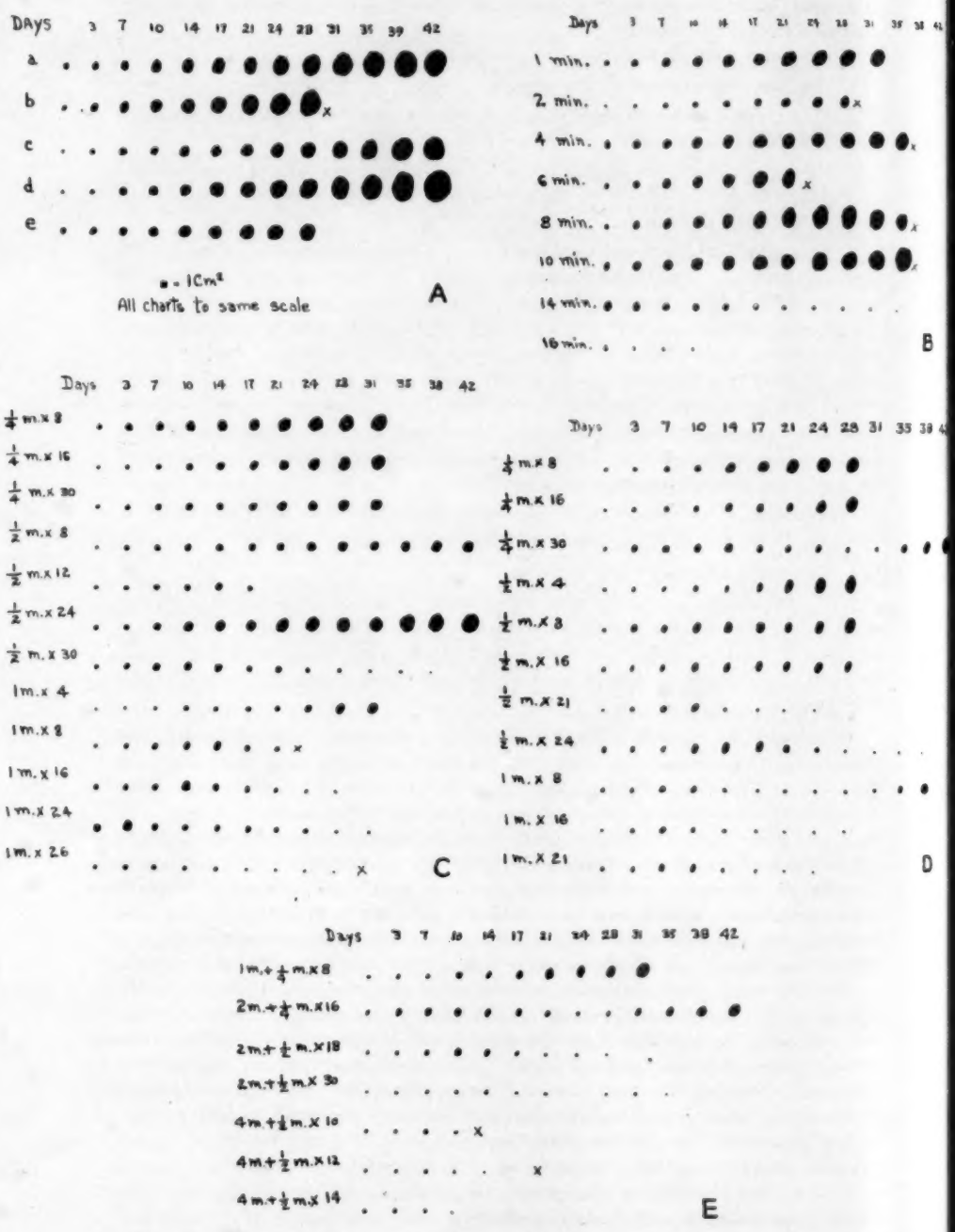

Fig. 1.—Flexner-Jobling carcinoma: *A*, untreated (controls); *B*, with single massive treatments; *C*, with daily fractional treatments; *D*, with daily subfractional treatments, and *E*, saturation treatments.

2. *Massive Doses (Fig. 1 B).*

Gross Observations.—Eight tumors were given single exposures (185 to 2,960 r) of the following durations, respectively: one, two, four, six, eight, ten, fourteen and sixteen minutes. Four others given single exposures of four, eight, eight and twelve minutes, respectively, were killed shortly afterward and the early changes studied. The tumors that received from one to ten minutes' irradiation continued to grow until several of them killed their host. The two tumors that received exposures of fourteen and sixteen minutes (2,590 and 2,960 r), respectively, failed to grow and eventually disappeared. The lethal massive dose for the Flexner-Jobling carcinoma is therefore 2,590 r. The rats that received the heaviest doses were very irritable, and one that received twelve minutes' irradiation died two days later of bronchopneumonia. After three weeks there were erythema and partial epilation of the irradiated skin in those that received the heaviest doses.

Histologic Observations.—One hour after four minutes' irradiation (740 r) no changes could be seen in the tumor except moderate hyperemia. One hour after eight minutes' irradiation (1,480 r), in addition to hyperemia, only slight swelling of many of the tumor cells could be seen. The tissues were fixed in solution of formaldehyde so that the finer cytologic details, such as mitochondria and Golgi bodies, were not studied. These have been admirably worked out by Ludford.[3] Two days after twelve minutes' irradiation one found occasional multinucleated giant cells, which seemed to have been formed by the fusion of several tumor cells (changes in surface tension). There was also a distinct increase in mitotic figures (4 or 5 per high power field), some of which were abnormal. There was no hyperemia, but a slight infiltration by polymorphonuclear leukocytes was visible in the connective tissue between the groups of tumor cells.

In the tumor fixed one week after eight minutes' irradiation (figs. 2 *B* and 3 *B*) many of the tumor cells were seen to be undergoing a characteristic primary degenerative change of a type which has been especially well described by Ludford.[3] In the nuclei of these cells the chromatin aggregated into large deeply staining clumps, which broke up and became scattered through the cytoplasm. This process of pyknosis and fragmentation was different in character from that which occurred in the areas of necrosis at the centers of the control tumors. The cytoplasm meanwhile became deeply eosinophilic and granular. The degenerated tumor cells were phagocytosed by polymorphonuclear leukocytes, which infiltrated the tumor heavily. In the tumor fixed two weeks after eight minutes' irradiation (fig. 3 *A*) most of the tumor cells had degenerated and only occasional islands of refractory tumor cells were left. The same degenerative changes as seen in the previous tumor were present here. In addition, there was extensive proliferation of collagenous connective tissue, which had replaced most of the tumor and separated the scattered islands of tumor cells that were left. In each of the two rats in which the tumor disappeared under irradiation (fourteen and sixteen minutes) there was found at the site of the tumor at six weeks, a very small calcified nodule surrounded by a fibrous tissue scar. The overlying skin showed atrophy but was otherwise intact. The skin of the other animals showed no changes at six weeks. The other organs in this series of animals showed no histologic changes.

3. *Fractional Doses (Fig. 1 C).*

Gross Observations.—Twelve tumors were given daily fractional doses as follows: three were given daily one-fourth minute treatments for eight, sixteen and thirty days, respectively; four daily one-half minute treatments for eight, twelve, twenty-four and thirty days, respectively, and five, daily one minute treatments for

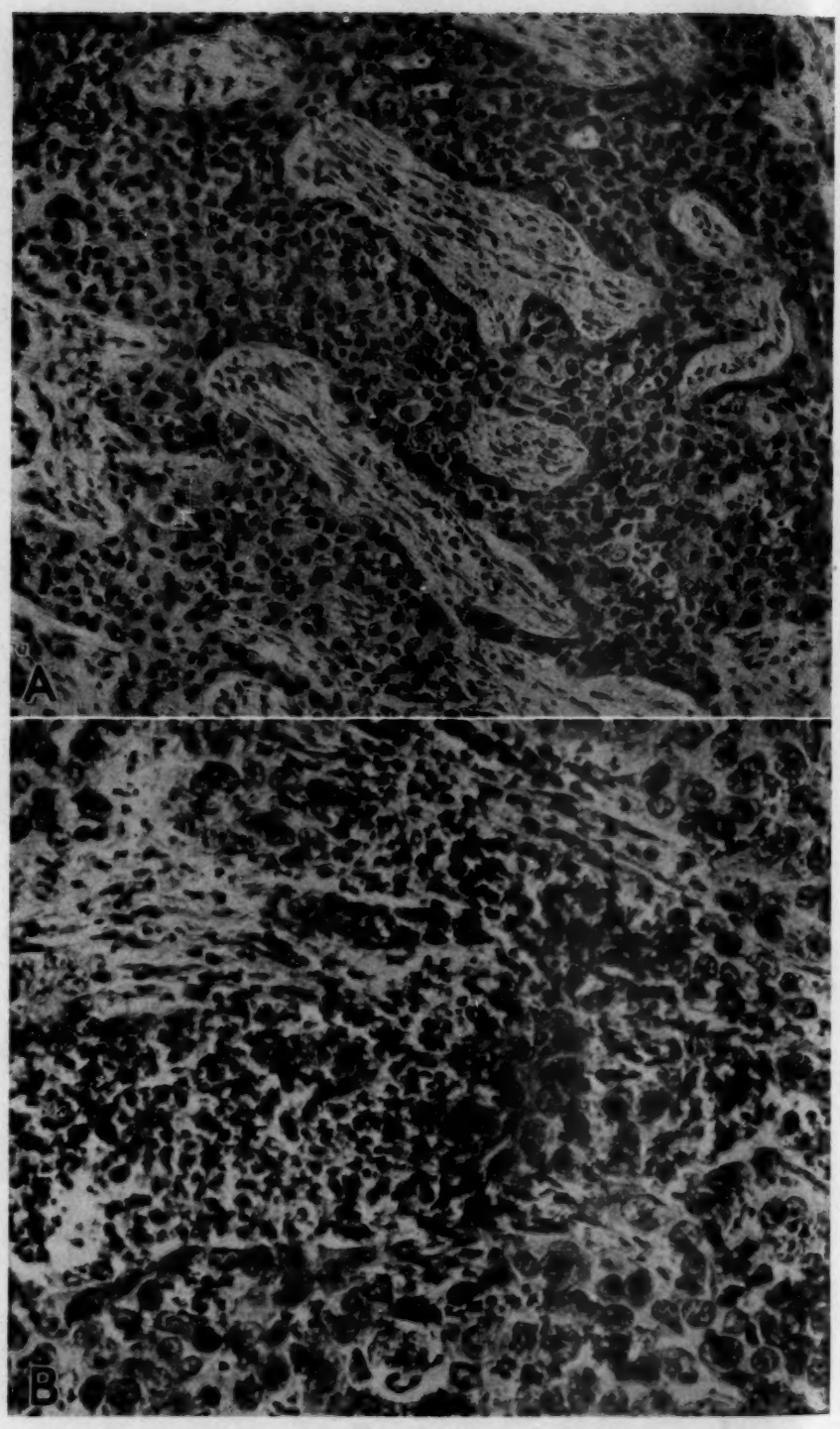

Fig. 2.—Flexner-Jobling carcinoma: *A*, untreated control at six weeks; × 200. The histologic appearance shown was constant in untreated tumors. *B*, tumor one week after an eight minute massive dose; × 300. Note the degenerative changes in some of the tumor cells and the infiltration by polymorphonuclear leukocytes. The tissues shown in this and subsequent figures were prepared by the hematoxylin-eosin method.

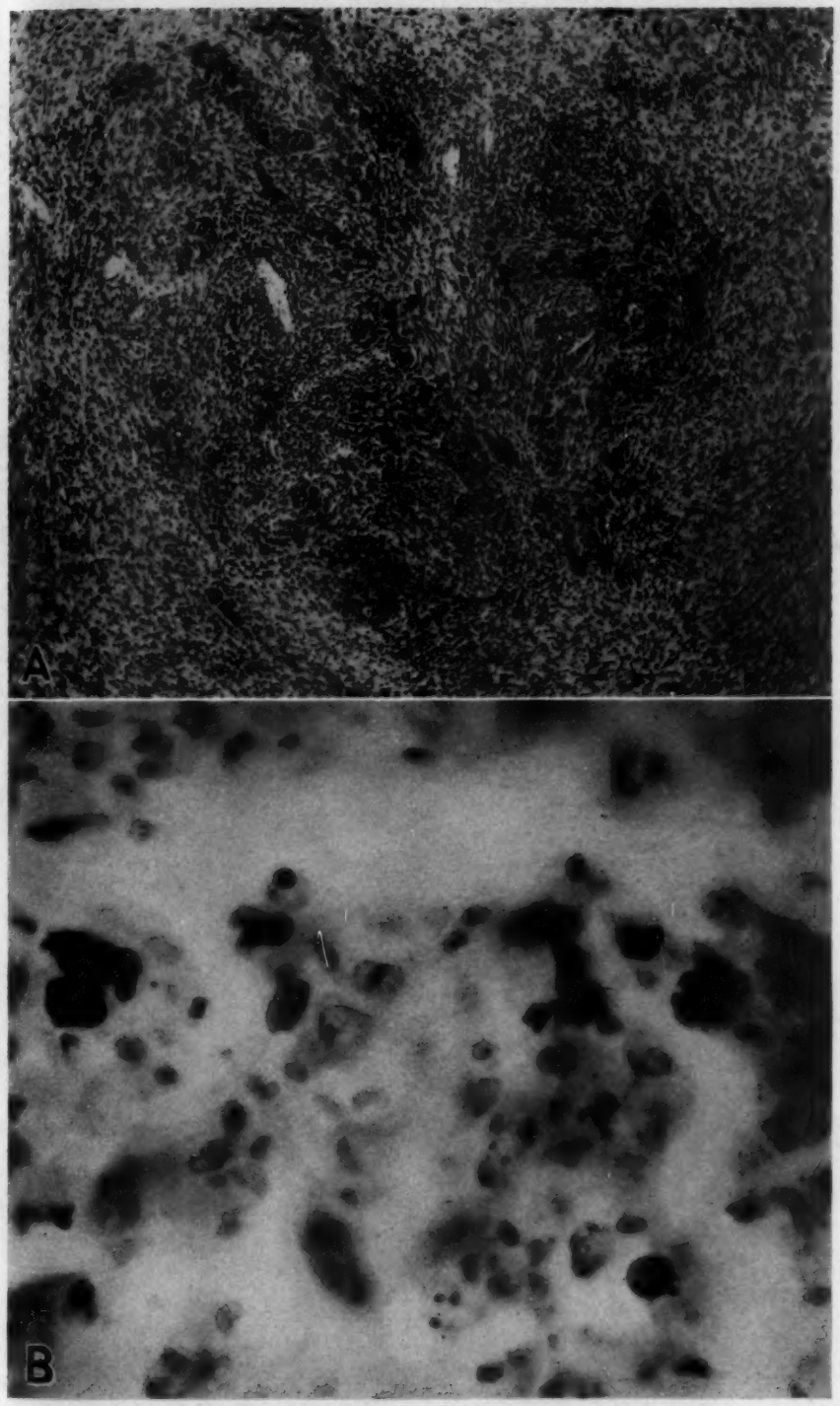

Fig. 3.—Flexner-Jobling carcinoma: *A*, two weeks after an eight minute massive dose; × 125. Groups of refractory tumor cells are left, surrounded by proliferating connective tissue. *B*, high power view of a field in *2 B*, showing deails of the primary degenerative changes in the tumor cells; × 600.

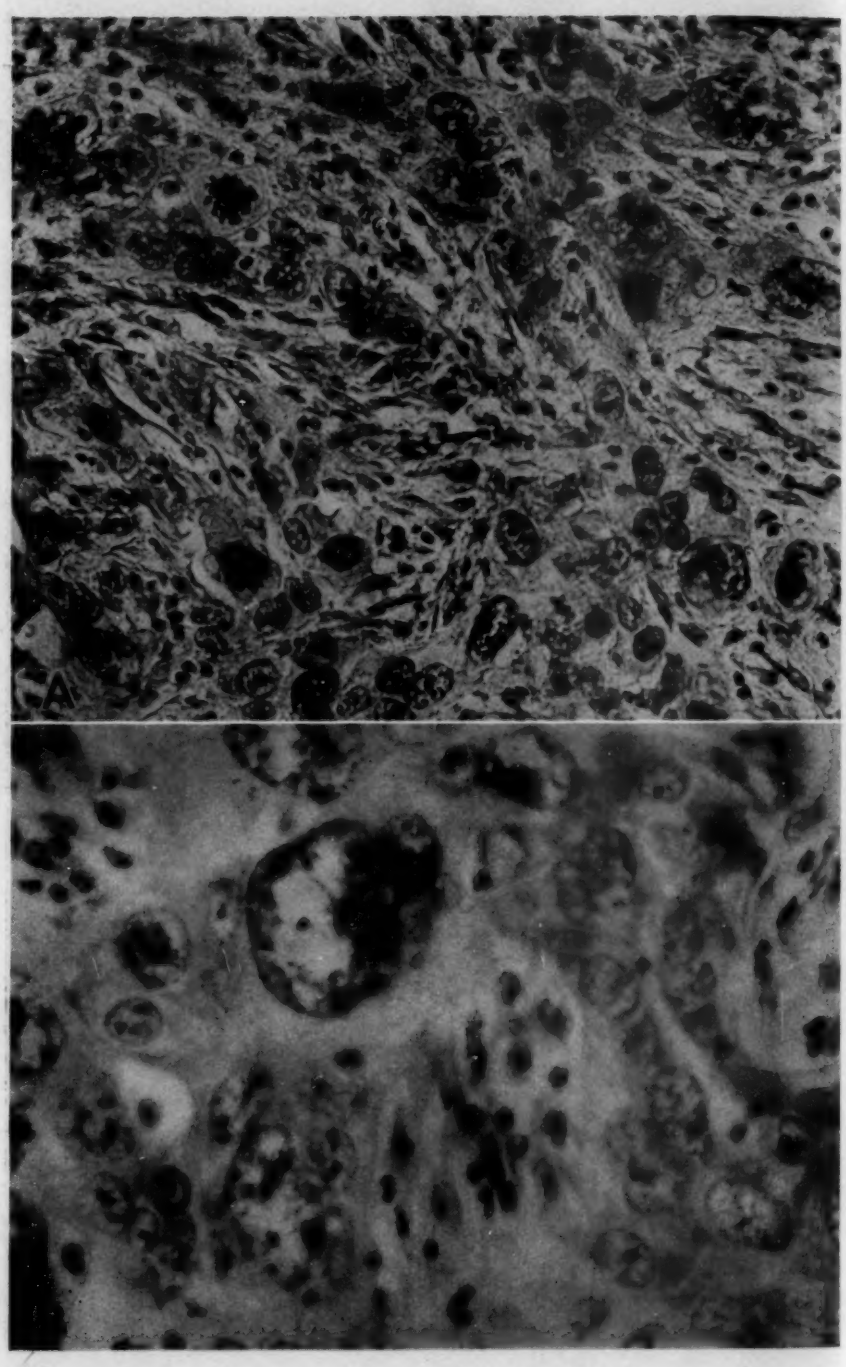

Fig. 4.—Flexner-Jobling carcinoma: *A*, at six weeks, after twenty-four daily fractional treatments of one-half minute each; × 300. Moderate pleomorphism may be seen. *B*, high power view of a field in *A*; × 600.

four, eight, sixteen, twenty-four and twenty-six days, respectively (total doses, from 370 to 4,810 r). Two other rats were killed after the tumors were given two treatments each of one-fourth and one-half minute. The tumors that received one-fourth minute's irradiation (46¼ r) daily were not checked but continued to grow, although at a less rapid rate than the controls. (In this respect the Flexner-Jobling carcinoma differs from the Jensen sarcoma, as tumors of the latter type that received one-fourth minute's irradiation daily grew slightly faster than the controls.) The tumors that received one-half minute's treatment daily increased in size initially and then were checked and either remained stationary to the end of the experiment or resumed growth, though slowly, at the end of the series of treatments. The tumors that received one minute of treatment daily (185 r) were likewise distinctly checked in their growth after an initial increase lasting a few days. The three tumors treated for one minute on sixteen, twenty-four and twenty-six days, respectively, disappeared.

With the fractional method, therefore, the lethal dose for the Flexner-Jobling carcinoma is 2,960 r at the rate of 185 r daily. This is slightly more than the lethal massive dose. An interesting difference between the two is that with the massive application the growth of the tumor is checked from the start, whereas with the fractional method there is a period of from seven to ten days during which the tumor continues to grow before it is checked.

Histologic Observations.—The two tumors examined immediately after two daily treatments of one-fourth and one-half minute showed no difference from the controls. The two tumors that received a one-fourth minute treatment on each of eight and sixteen days, respectively, showed no difference from the controls at four weeks except a slight increase in abnormal mitotic figures and giant cells. The tumor that received daily one-fourth minute treatments for thirty days showed at four weeks about the same slight increase in abnormal mitoses and giant cells and also moderate proliferation of connective tissue; in addition, in the central necrotic area, there were extensive deposits of calcium. The two tumors that were irradiated for one-half minute on eight and twelve days, respectively, bore a strong resemblance to that which received one-fourth minute's treatment daily for thirty days. The tumor receiving a daily half minute treatment for twenty-four days had at six weeks a different appearance (figs. 4 *A* and *B*). In addition to primary degenerative changes as seen after massive doses, there was distinct pleomorphism of the tumor cells. There were many abnormal cell forms, especially giant cells with hyperchromatic nuclei, some of which were in mitosis. Centrally located deposits of calcium were also present. The tumor which received one-half minute of treatment daily for thirty days had practically the same structure except that the pleomorphism was even more marked (fig. 5 *A*). The tumor that received four daily exposures of one minute each started to grow again about three weeks after the end of the treatments. Fixed a week later, it showed a structure much like that of a young untreated tumor except for marked increase in the number of giant cells.

The tumor that received eight daily irradiations of one minute each and was fixed in solution of formaldehyde two weeks later revealed a histologic picture different from those described. It was one of six in which giant cell formations were striking. (Figures 5 *B* and 6 *A* show the most marked formations of this type, to be described later.) The tumor presented a moderately advanced stage of growth. Although about a fourth of the cells in the section had the appearance of the cells of the controls, the majority were mononuclear giant cells four or five times the size of the others. These cells varied from round to oval and had

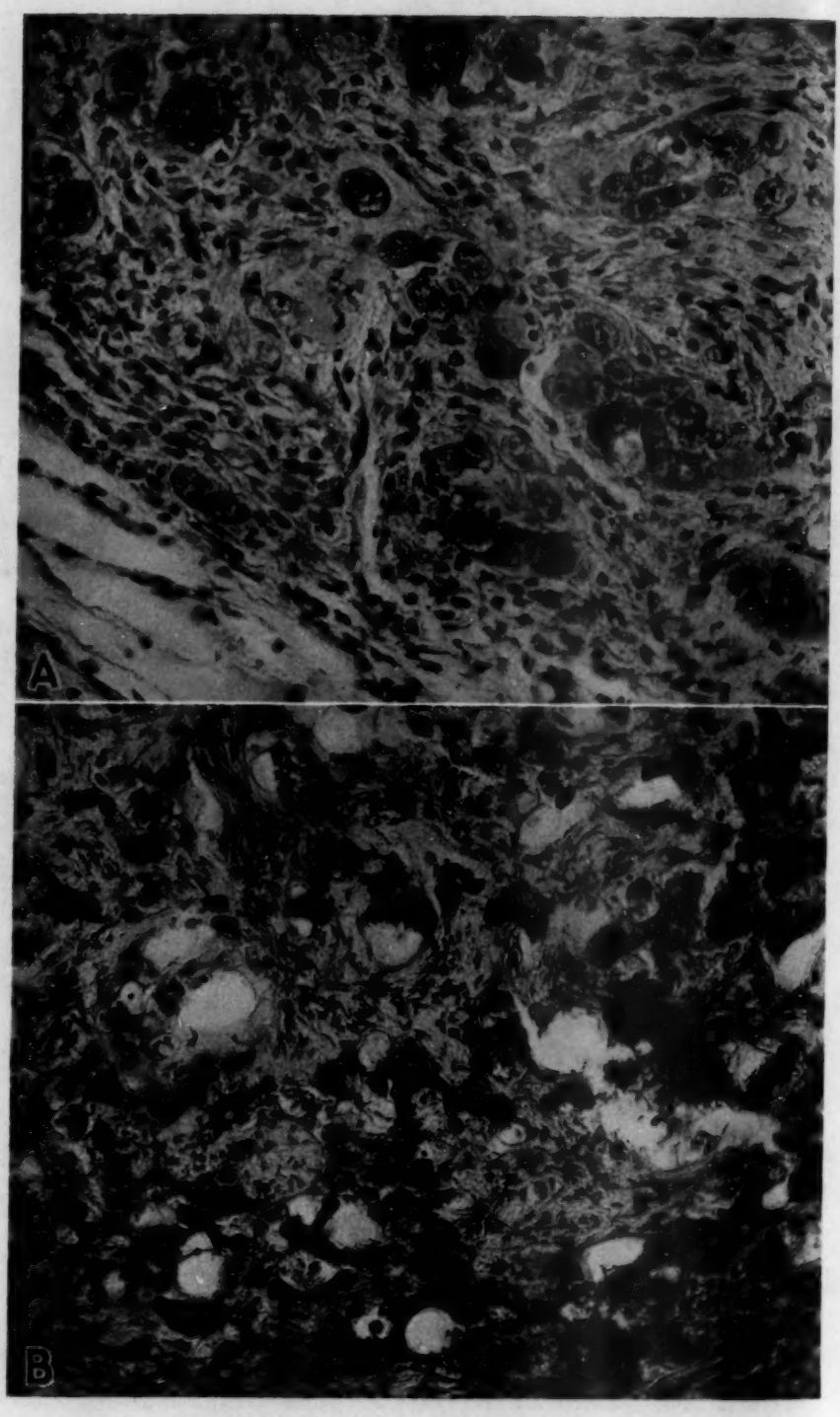

Fig. 5.—*A*, Flexner-Jobling carcinoma at six weeks, after thirty daily fractional treatments of one-half minute each; × 300. Here pleomorphism is more advanced. *B*, Flexner-Jobling carcinoma at four weeks, after sixteen daily treatments of one minute each, subfractionized; × 125. Marked pleomorphism is shown. The tumor consists of an almost pure growth of giant cells.

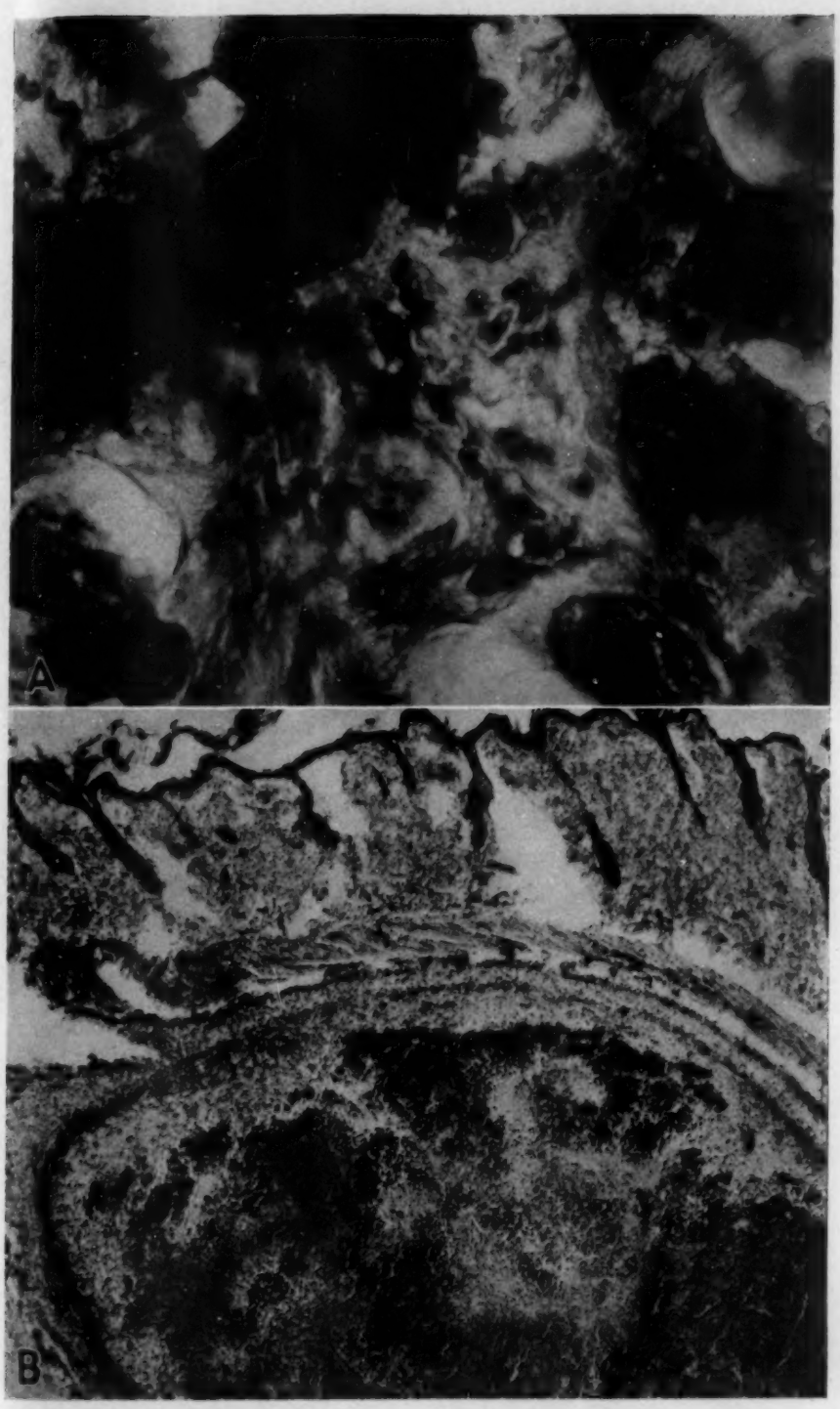

Fig. 6.—*A*, high power view of a field in figure 5 *B*; ×600. *B*, Flexner-Jobling carcinoma after saturation treatment—i. e., a four minute treatment plus twelve daily half minute treatments; ×25. The center of the tumor is necrotic. The refractory tumor cells which have proliferated at the periphery have become transformed into giant cells, the nuclei of which have calcified.

abundant very palely staining cytoplasm, which was often vacuolated. In many instances the vacuoles in a cell had coalesced so that a single large vacuole was seen pushing the nucleus to one side, giving the cell the appearance of a signet ring. The nuclei of these cells were stained deeply, varied from round to oval and sometimes were indented; the nuclear membrane was distinct but not thickened, and the chromatin network was dense and composed of coarse granules. From one to three large prominent nucleoli, usually round but sometimes irregular in outline, were present. Many of these cells were in mitosis; the mitotic figures were abnormal, consisting of a great many swollen club-shaped chromosomes, often scattered unevenly throughout the cell. The nuclei of these giant cells were seen to undergo calcification. In this tumor all the stages up to complete calcification were visible. The larger nuclei became deeply basophilic, and scattered granules of calcium began to appear within them. One got the impression that the coarser aggregates of chromatin became calcified. As the calcium deposits increased in amount, the nucleus shrank slightly. Finally the entire nucleus was transformed into a round or oval calcified body. Masses of such discrete calcified nuclei were scattered throughout the tumor. The three tumors that were irradiated for one minute daily on sixteen, twenty-four and twenty-six days, respectively, disappeared. At the site of the tumor small nodules of calcium were found, surrounded by dense connective tissue. The rats that received one minute's treatment daily for twenty-four and twenty-six days, respectively, did not grow as well as the other rats. At the end of the experiment they weighed only 45 and 60 Gm., contrasted with 90 to 120 Gm. for the rest. The overlying skin in both of these was somewhat atrophic but intact. Histologic examination of the bone marrow in the latter animal revealed it to be composed of a loose myxomatous connective tissue with practically no areas of hematopoiesis, like the bone marrow in aplastic anemia. The spleen of this rat showed moderate hemosiderosis. The other organs revealed no change. The skin and other organs of the other rats of this series showed no histologic changes.

4. *Subfractional Doses (Fig. 1D).*

Gross Observations.—In this series a modified form of protraction was devised in order to keep all the factors in treatment constant, as explained already. Each fractional treatment was subdivided into five or ten second fractions separated by intervals of two minutes, so that the total duration of each daily treatment was spread over from six to twelve minutes. Eleven tumors were given the following treatments: three, one-fourth minute daily in subfractions for eight, sixteen and thirty days, respectively; five, one-half minute daily in subfractions for four eight, sixteen, twenty-one and twenty-four days, respectively and three, one minute daily in subfractions for eight, sixteen and twenty-one days, respectively. In addition, two tumors were fixed immediately after two daily treatments of one-fourth minute and one minute subfractionated. This series of tumors largely parallels the previous series. Practically all the tumors reacted in the same manner as those given fractional doses. With daily doses of one-fourth minute subfractionized, the tumors continued to grow at a rate slightly less than that of the controls. The half minute daily treatments had more effect; although four or eight daily half minute treatments, subfractionized, did not check the tumor appreciably, sixteen daily half minute treatments, subfractionized, inhibited growth. Two tumors were destroyed by half minute treatments given in subfractions daily for twenty-one and for twenty-four days (1,943 r and 2,220 r, respectively; these doses are less than the lethal massive dose of 2,590 r). One minute's treatment given in subfractions daily for eight days held the tumor in

check, but growth was resumed later. One minute's treatment given subfractionally daily for sixteen days in one case and for twenty-one days in another destroyed the tumors.

Histologic Observations.—The microscopic picture of this series of tumors paralleled that of the previous series. At four weeks the tumors that received the shorter series of daily subfractionized one-fourth minute exposures differed from the controls only in the greater number of giant cells visible. The tumor which received one-fourth minute in subfractions daily for thirty days was extensively necrotic in the center at six weeks; at the periphery was a narrow zone of tumor tissue containing many giant cells. In this tumor the various stages of calcification of giant cells were again seen clearly. The tumor receiving a half minute in subfractions daily for four days was much like the controls except for numerous giant cells. The tumor receiving a half minute in subfractions daily for eight days resembled the one which received one-fourth minute in subfractions daily for thirty days in having large numbers of giant cells which showed the various stages of calcification.

The next tumor, which received a half minute in subfractions daily for sixteen days and was fixed in solution of formaldehyde at four weeks, was the most striking in the entire series (figs. 5*B* and 6*A*). There was a complete change in the character of the tumor. It was composed of practically a pure culture of huge hyperchromatic giant cells. These cells answered to the same description as the giant cells in the tumor of the previous series that resembled this one (one minute's treatment daily for eight days). The process was more extensive than in the previous tumor; practically all the cells had been transformed into a race of abnormal giant cells, with only here and there a normal tumor cell left. The process of gradual calcification of the nuclei of the giant cells was clearly seen in this tumor also. The next two tumors (a half minute in subfractions daily for twenty-one and twenty-four days, respectively) disappeared under the treatments, and at six weeks small nodules of calcium were found at the tumor sites. In the first of these (a half minute in subfractions daily for twenty-one days) there were seen at the periphery of the nodule of calcium a few deeply basophilic giant nuclei that appeared still viable; one had the impression that a few more treatments would have been necessary to destroy the tumor completely. The tumor receiving one minute in subfractions daily for eight days regrew toward the end of the experiment and was composed of active tumor tissue containing many giant cells. The last two tumors of this series (one minute in subfractions daily for sixteen and twenty-one days, respectively) disappeared completely under the treatment. In the last two tumors the overlying skin was partially epilated and histologically slightly atrophic at six weeks. These two rats did not grow well (80 and 65 Gm.), but histologic examination of the various organs, including the bones and bone marrow, showed nothing abnormal.

5. *Saturation Doses (Fig. 1E).*

Gross Observations.—Seven tumors received a form of "saturation" treatment, consisting in a moderate massive dose on the first day, followed by small daily fractional treatments. One tumor received one minute treatment plus eight daily one-fourth minute treatments; one tumor, a two minute treatment plus sixteen daily one-fourth minute treatments; two tumors, two minute treatment plus eighteen and thirty daily half minute treatments, respectively, and three tumors, a four minute treatment plus ten, twelve and fourteen daily half minute treatments, respectively. One other tumor received a four minute treatment plus two daily half minute treatments and was fixed in soluion of formaldehyde immediately

afterward. The first tumor (given a one minute treatment plus eight daily one-fourth minute treatments) increased in size at a rate slightly less than that of the controls. The second (given a two minute treatment plus sixteen daily one-fourth minute treatments) continued to grow but was inhibited after two weeks; at the end of the series of treatments it began to grow again. All the other tumors in this series disappeared under the treatments. Two of the animals died of bronchopneumonia just before the tumors disappeared, furnishing two more valuable tumors for histologic study. In the five tumors that disappeared it is important to point out two interesting features: First, the tumors were inhibited in their growth from the beginning (with one exception, that given a two minute treatment plus eighteen daily half minute treatments); i. e., there was practically no increase in size during a period of a week or ten days as with the other two divided dose methods. Second, in four of the five tumors the total dose necessary to destroy the tumor was less than the lethal massive dose (1,665 r, 1,850 r, 2,035 r and 2,035 r, as against 2,590 r, the lethal massive dose). Contrasted with the effective destruction of the tumors was the absence of injury in either the skin or the host. Only in the last tumor (given a four minute treatment plus fourteen daily half minute treatments) was there slight epilation of the skin, and the animal did not grow quite as well (85 Gm.).

The results in this series seem to speak for a practical advantage in this method. The initial moderate massive dose inhibits the growth of the tumor (important in fast growing tumors), and the succeeding daily fractional doses destroy it, with less total radiation (in this experiment) than with other methods.

Histologic Observations.—The tumor that received a four minute treatment plus two daily half minute treatments showed only an increase in mitoses. The first two tumors of the treated series showed the microscopic structure of regrowths, already described. The tumors in the animals that died just before the final disappearance of the tumors were very interesting (figs. 6 *B*, 7 *A* and *B*). Sections showed practically no viable tumor cells left. The center of the tumor consisted of necrotic tissue, no doubt derived from the bulk of the tumor cells, which had undergone primary degenerative changes, i. e., the more sensitive cells that had been destroyed by the moderate massive dose. In the periphery of the tumor, however, one saw a different picture. Embedded in a fairly dense connective tissue stroma were masses of discrete round or oval calcium bodies, the calcified nuclei of giant cells. The refractory tumor cells which survived the moderate massive dose had attempted to proliferate at the edge of the tumor, near the vascular supply. Under the influence of the subsequent fractional irradiation, they finally became transformed into abnormal giant forms, which calcified. The tumors that disappeared completely were represented only by small calcified nodules at the tumor sites (fig. 8 *A*).

JENSEN SARCOMA

1. *Untreated Controls (Fig. 9 A).*

Gross Observations.—Seven rats with tumors of this type were left untreated as controls. Three were killed at ten days for histologic study. In the other four animals the tumors increased in size at a rapid rate. Two of the tumors killed the animals by invasion and metastases. One rat was killed at four weeks and the other at six weeks for histologic study.

Histologic Observations.—The Jensen sarcoma is a small spindle cell sarcoma (fig. 8 *B*). In these rats, at ten days, the tumor was composed of interweaving

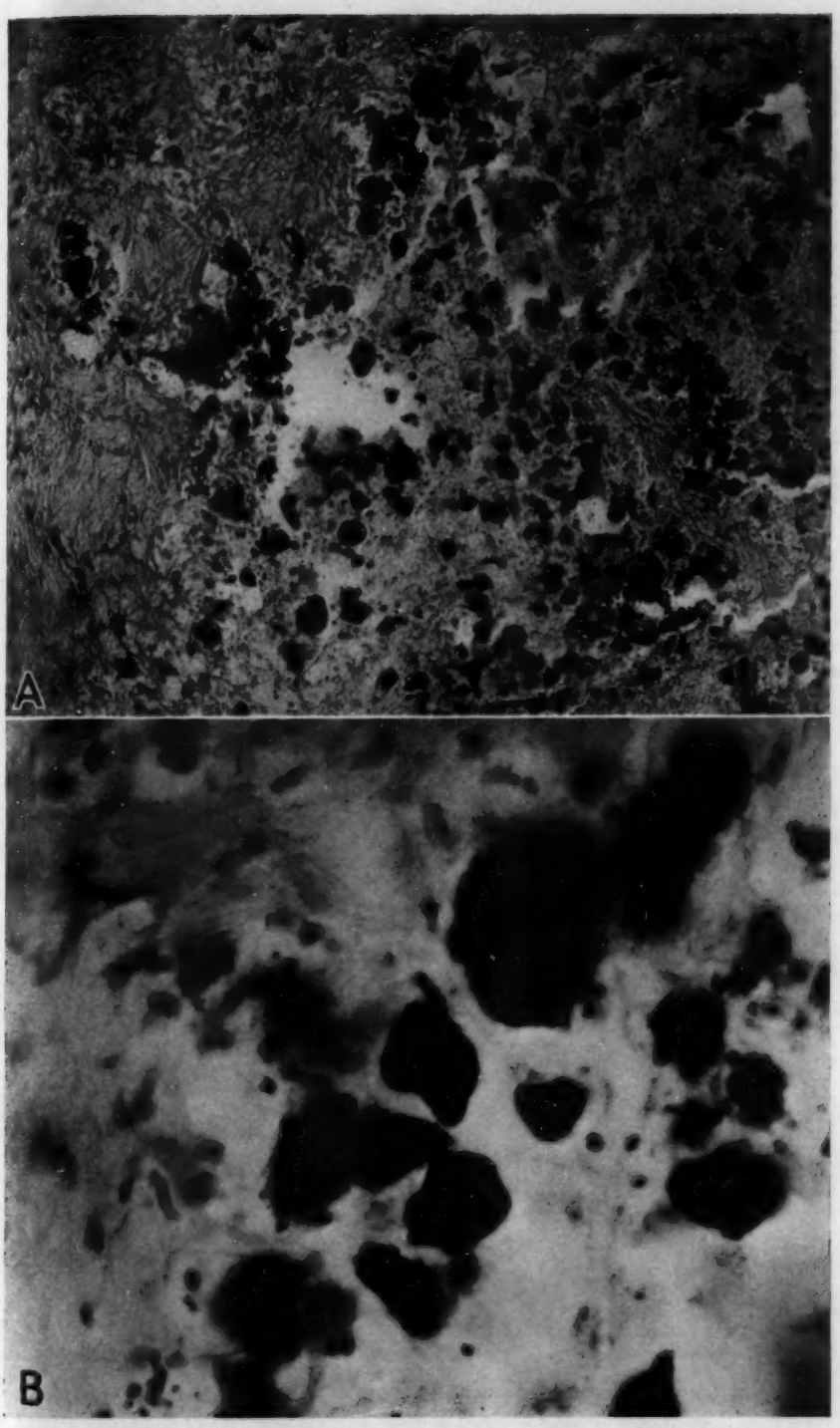

Fig. 7.—Flexner-Jobling carcinoma: *A*, medium low power view of the peripheral zone of 6 *B*; × 200. *B*, high power view of a field in *A*, showing the details of the calcified nuclei of giant cells; × 600.

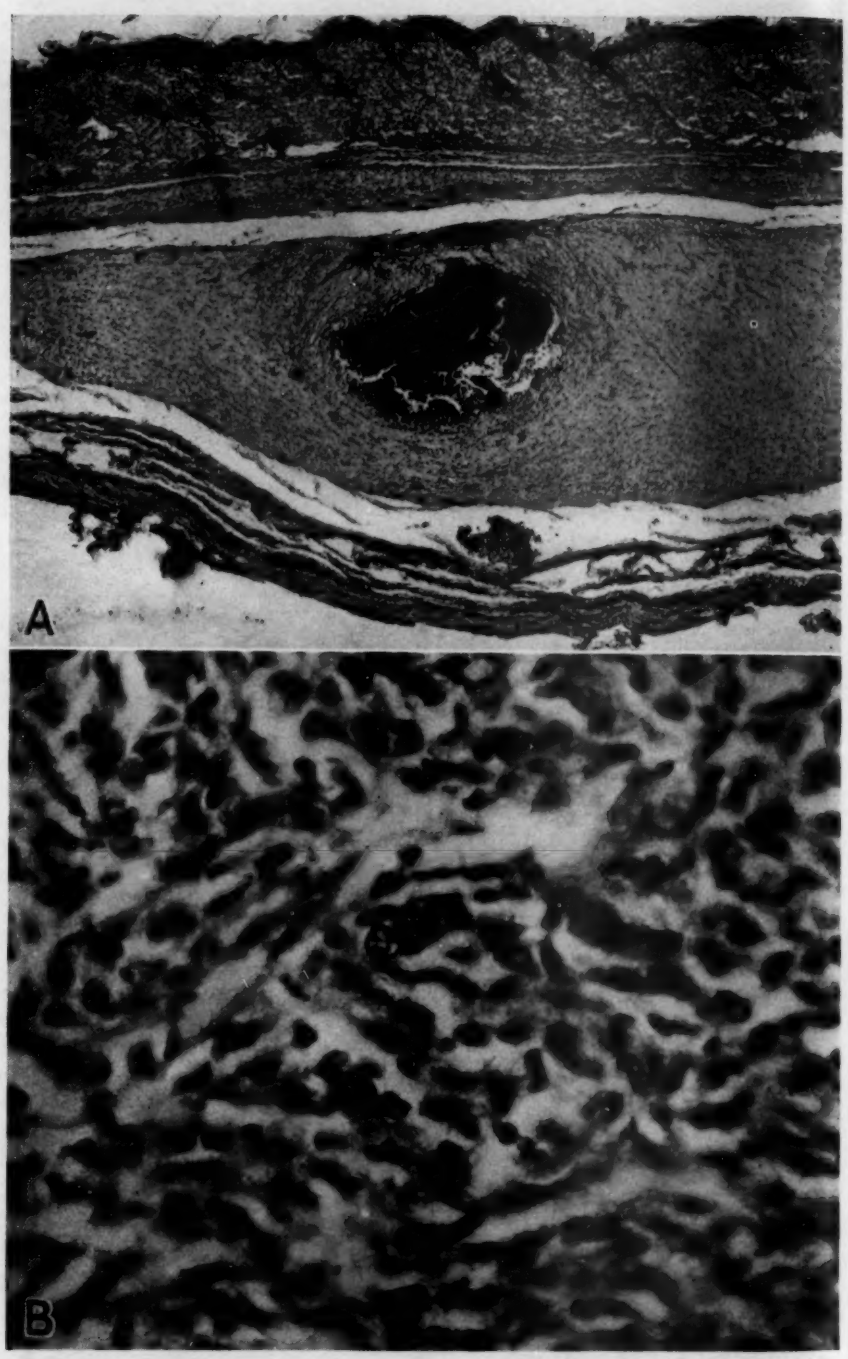

Fig. 8.—*A*, Flexner-Jobling carcinoma at six weeks, after saturation treatment—a four minute irradiation plus twelve daily half minute irradiations; × 25. Only a small nodule of calcium is left, surrounded by connective tissue. *B*, Jensen sarcoma, an untreated control, at ten days; × 600. This histologic picture is constant in untreated tumors.

bundles of fusiform cells with a fair amount of homogeneous cytoplasm that stained yellow by the Van Gieson method. There were no collagen fibers except a very few delicate ones located about the small capillaries which were scattered through the tumor. The nucleus of the tumor cell was oval; it had a distinct nuclear membrane and chromatin in the form of a compact net of fine granules. One or two nucleoli were usually visible. Many mitotic figures, occasionally abnormal, were seen. The cells and nuclei varied only slightly in size. The tumor was surrounded by a narrow layer of connective tissue containing a few lymphocytes and a number of dilated blood vessels. At four weeks and also at six weeks there was no change in the character of the tumor cells or in that of the tumor except that the tumor was much larger and there was a large area of central necrosis without calcification, surrounded by intact tumor.

2. *Massive Doses (Fig. 9 B).*

Gross Observations.—Eight tumors were given irradiations of the following durations: one, two, two, four, four, four, eight and twelve minutes (from 185 to 2,220 r), respectively. Those that received one and two minutes' irradiation continued to grow at a rapid rate and killed the animals. These tumors seemed to grow somewhat faster than those in the controls; whether this was evidence of stimulation or pseudostimulation, we cannot say. Four minutes' irradiation or more invariably destroyed the tumor (a lethal massive dose of 740 r). Shrinkage of the tumor was noticeable from the beginning, i. e., there seemed to be no period of latency after massive doses. The tumor that received twelve minutes' treatment disappeared rapidly, within six days. These findings are in accord with clinical experience in rapidly growing radiosensitive tumors. Four other tumors were fixed in solution of formaldehyde as follows: one hour after a two minute treatment, two days after a four minute treatment and five and seven days after an eight minute treatment, respectively.

Histologic Observations.—One hour after two minutes' irradiation there was marked hyperemia of the tumor, but no other changes. Two days after four minutes' irradiation there was a moderate amount of central necrosis, and the periphery of the tumor was infiltrated by polymorphonuclear leukocytes. Five days after eight minutes and seven days after eight minutes' exposure there was extensive necrosis with only a narrow zone of tumor cells left in the periphery. No abnormalities of the tumor cells were visible. However, the central necrotic tissue was extensively infiltrated by calcium; the central necrotic areas of the tumors in the controls contained no calcium. The tumors that received one and two minutes' irradiation grew rapidly and killed the animals within two weeks. Histologically they resembled the tumors of the controls except for slight deposits of calcium in the central necrotic areas. The tumors that disappeared completely with massive doses left no trace except small calcified nodules.

Abnormalities of the tumor cells of the Jensen sarcoma cannot be found after massive doses. Changes take place so rapidly in this fast growing tumor that such abnormal forms do not persist long. Some must be formed, however, as evidenced by the deposits of calcium in the necrotic zones. In a few of the tumors that received the long series of fractional treatments formation of giant cells with subsequent calcification was found, as will now be described.

3. *Fractional Doses (Fig. 9 C).*

Gross Observations.—Ten tumors were irradiated as follows: Three were given one-fourth minute on eight, fifteen and fifteen days, respectively; four were

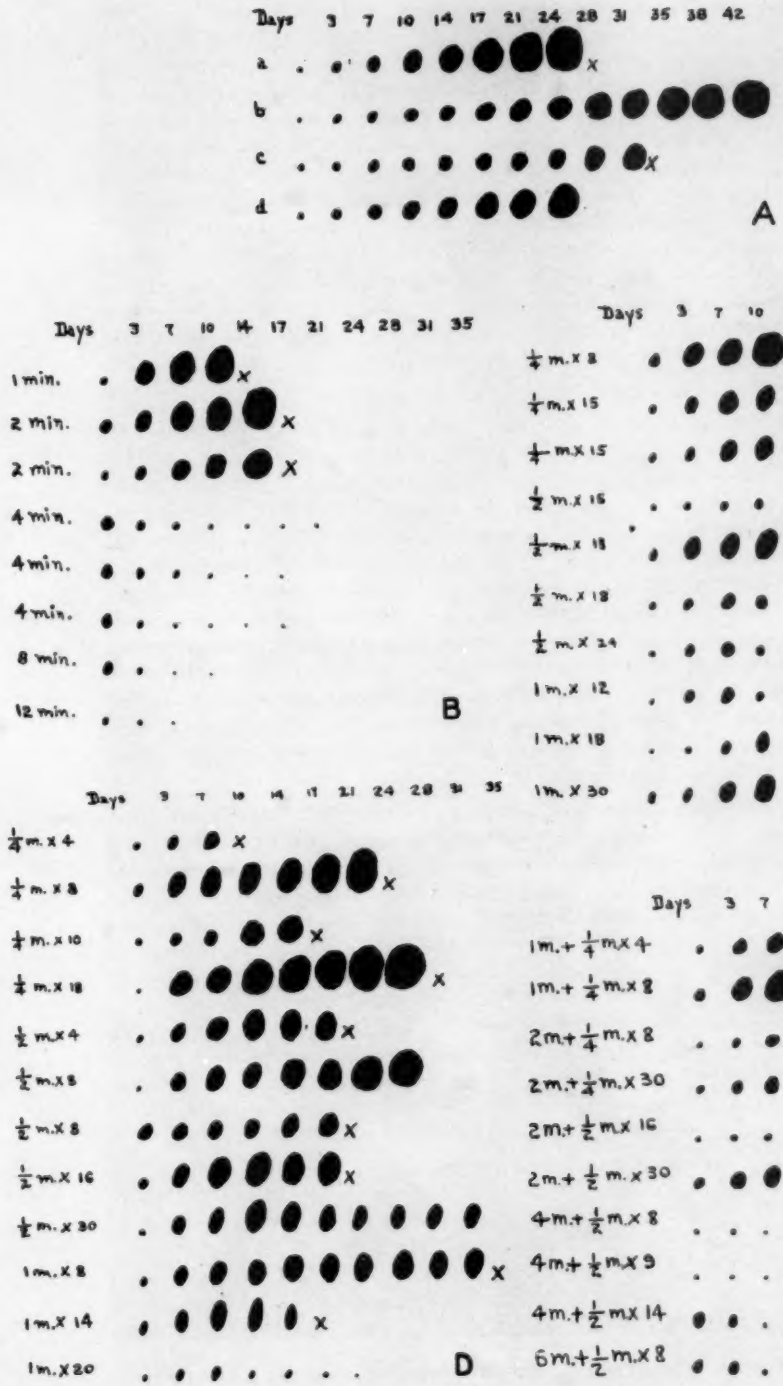

Fig. 9.—Jensen sarcoma: *A*, untreated (controls); *B*, with massive treatments; *C*, with fractional treatments; *D*, with subfractional treatments, and *E*, with saturation treatments.

given one-half minute on fifteen, eighteen, eighteen and twenty-four days respectively, and three were given one minute on twelve, eighteen and thirty days, respectively. Three other tumors were given short series of fractional doses. The first three, which received one-fourth minute's irradiation daily, grew rapidly and killed the animals within from two to three weeks. These also grew at a faster rate than did those in the controls, but again, whether this is evidence of stimulation of growth cannot be asserted, but the possibility must be seriously considered. Two of the tumors that were irradiated for one-half minute daily were inhibited in their growth after ten days and two weeks, respectively, but they had grown to such an extent that they killed the animals. Two other tumors that received the half minute daily treatments were inhibited within two weeks after an initial increase in size and were finally destroyed by continuing the daily treatments. The tumors that received one minute's treatment daily reacted in the same manner as the previous two. One minute daily was more effective in reducing the period of latency to from five to seven days, but the larger dosage was more injurious to the animals, two of them dying of bronchopneumonia. In the third (given a one minute treatment daily for thirty days) the skin overlying the tumor became epilated, and the growth of the rat was stunted.

Histologic Observations.—The three tumors that were fixed in a solution of formaldehyde immediately after a short series of fractional treatments resembled the tumors in the controls. The tumors that received one-fourth minute's irradiation daily grew very large; at two and three weeks they resembled the tumors in the controls microscopically except that there were deposits of calcium in the central necrotic areas. One had the impression that there was an increase in mitotic figures, but this could not be judged with accuracy.

The two tumors that were inhibited in their growth after being given a daily half minute treatment for fifteen and eighteen days, respectively (the rats died before the tumors disappeared), were of much interest histologically. In these tumors there was the same formation of giant cells with calcification of their nuclei as in the Flexner-Jobling carcinoma, but not nearly as pronounced. The tumors were small, the centers were occupied by necrotic material containing deposits of calcium, and there was a peripheral zone of intact tumor. The tumor cells in this peripheral area were distinctly pleomorphic, with many hyperchromatic giant cells (fig. 10 *A*). Although the picture was not striking, it was distinct and indicated a change in the character of the tumor cells. At the border between intact and necrotic tumor was a zone in which the nuclei of these cells were seen to undergo calcification (fig. 10 *B*). All the other tumors in this series disappeared under irradiation, only connective tissue scars with small calcified centers remaining.

4. *Subfractional Doses (Fig. 9 D).*

Gross Observations.—Twelve tumors in this series received the following treatments: four, one-fourth minute, subfractionized, daily for four, eight, ten and eighteen days, respectively; five, one-half minute, subfractionized, daily for four, eight, eight, sixteen and thirty days, respectively; three, one minute, subfractionized, daily for eight, fourteen and twenty days, respectively. The results in this series were very poor. One-fourth minute doses, subfractionized, allowed the tumors to grow more rapidly than those in the controls, and the animals died of invasion. One-half minute doses, subfractionized, inhibited the tumors only slightly after about two weeks. Most of these tumors continued to grow and

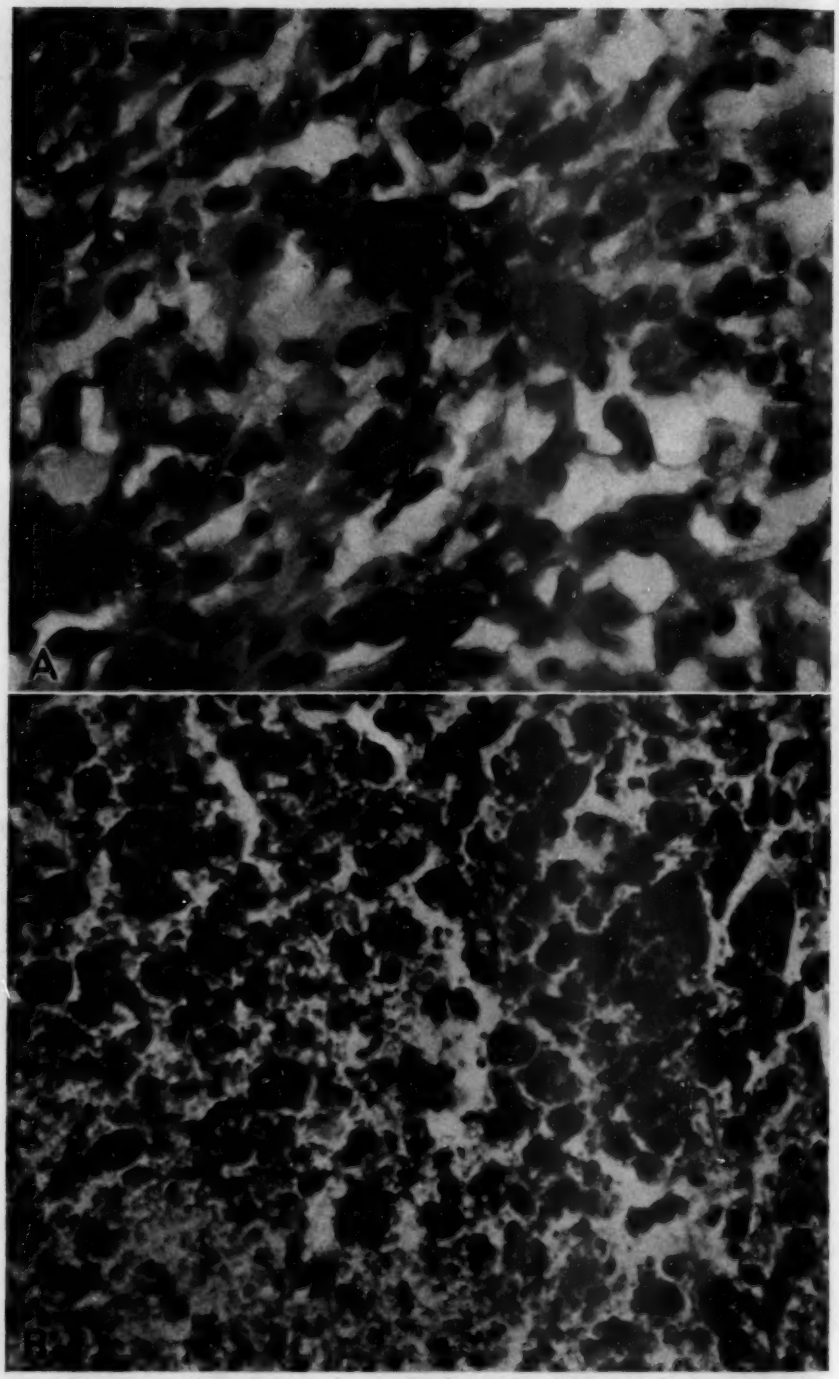

Fig. 10.—Jensen sarcoma: *A*, after fifteen daily fractional treatments of one-half minute each; × 600. Moderate pleomorphism may be noted. *B*, a different field in the tumor shown in *A*; × 600. Note the calcification of the nuclei of giant cells.

killed their hosts. Subfractionized one minute doses were only slightly more effective; in two cases the tumors grew large enough to kill the animals; however, a tumor given a subfractionated one minute dose daily for twenty days disappeared under the treatment.

The poor results in this series contrast with the better results of simple fractioning. There were slightly better results by this method, however, in the Flexner-Jobling carcinoma. The conclusion is justified that there is no essential difference between subfractional (i. e., protracted fractional) doses and simple fractional doses.

Histologic Observations.—Microscopic examination of the tumors in this series revealed no differences between them and those of the previous series. There was the same central necrosis of the tumors with occasional appearance of pleomorphism, i. e., giant cells the nuclei of which underwent calcification. Four other tumors which received very short series of subfractional doses revealed no differences from the controls.

5. *Saturation Doses (Fig. 9 E).*

Gross Observations.—The saturation method was used on ten tumors as follows: Two tumors received a one minute treatment plus daily one-fourth minute treatments for four and eight days, respectively; two tumors, a two minute treatment plus daily one-fourth minute treatments for eight and thirty days, respectively; two tumors, a two minute treatment plus daily one-half minute treatments for sixteen and thirty days, respectively; three tumors, a four minute treatment plus daily one-half minute treatments for eight, nine and fourteen days, respectively, and one tumor, a six minute treatment plus daily one-half minute treatments for eight days. The effective results of this method in this series are striking. The first two tumors in the series, receiving very weak doses (a one minute treatment plus daily one-fourth minute treatments) grew in size until they killed the hosts, but all the rest of the tumors were destroyed by the treatments. A two minute initial treatment inhibited the tumors after a period of latency of about a week, and subsequent daily treatments destroyed them. One of these was fixed in solution of formaldehyde before its final disappearance. A four minute initial treatment inhibited growth of the tumors with no period of latency. Subsequent daily half minute treatments effectively destroyed the tumors within a comparatively short time, less time than was required by the four minute massive dose alone. A six minute initial treatment was even more effective. Two of the rats in this series died of bronchopneumonia. Three other tumors received a short series of weak saturation doses for histologic study.

Histologic Observations.—All the tumors treated with weak saturation doses showed no difference from those in the other series treated with weak doses. The tumors that disappeared left only a fibrocalcareous scar. The tumor that received an initial two minute treatment plus eight daily one-fourth minute treatments and was fixed in solution of formaldehyde a week later showed pleomorphism and calcification of the giant cells, similar to those described.

WALKER TUMOR (FIG. 11 A)

Only ten Walker tumors were studied. Two of these, in controls, were not irradiated. An eight minute massive dose failed to destroy the tumor. One-fourth or one-half minute daily fractional doses failed to stop growth. A one minute daily dose inhibited growth, but the rat died after eight exposures. One-fourth or one-half minute daily doses, subfractionized, failed to inhibit growth. A one

minute daily dose, subfractionized, destroyed the tumor after nineteen treatments, but the rat died.

The Walker tumor is a slow growing radioresistant tumor. Histologically it is a carcinosarcoma (fig. 12*A*). The tumor is chiefly a fibrosarcoma with a moderate

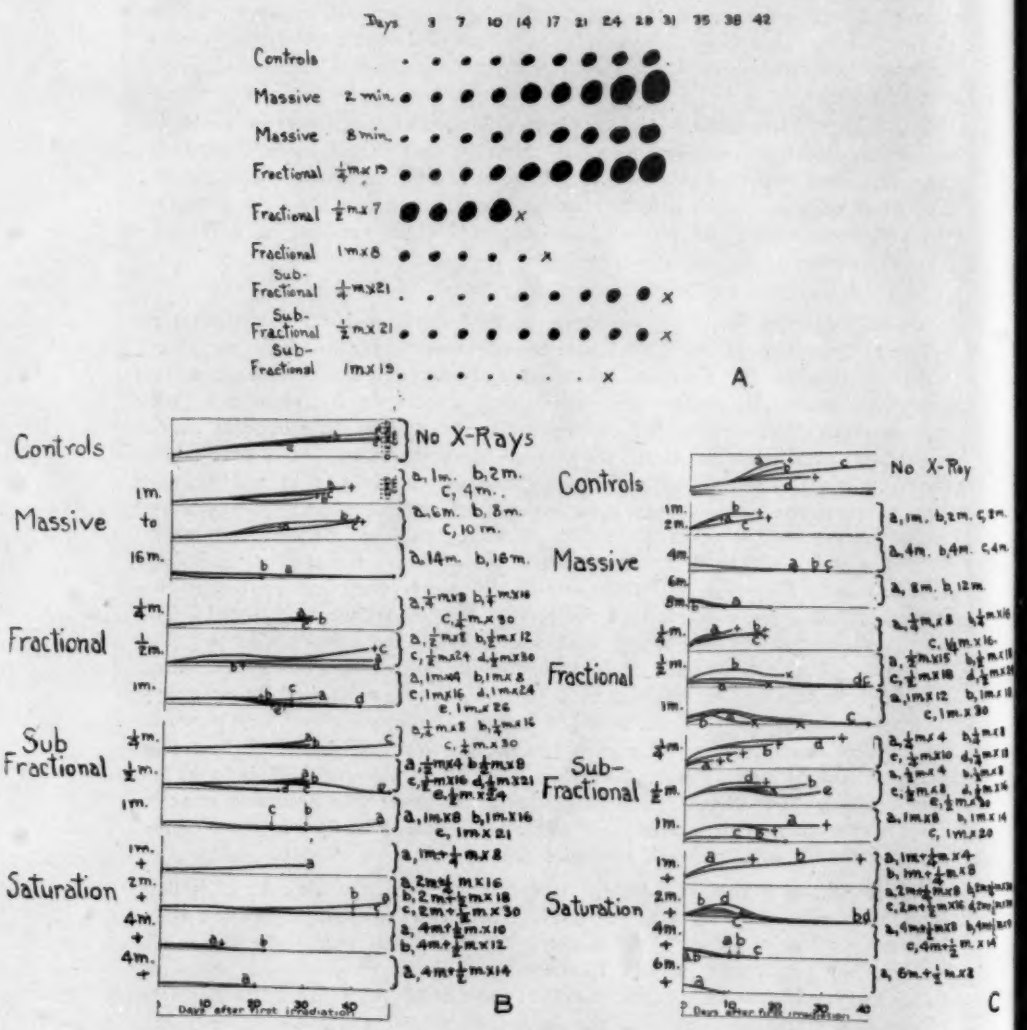

Fig. 11.—*A*, Walker tumor; *B*, Flexner-Jobling carcinoma, summary of all groups; *C*, Jensen sarcoma, summary of all groups.

amount of collagen, and here and there are scattered islands of epithelioid cells. A change in character under irradiation was observed in only one of these tumors; it had received twenty-one daily half minute doses, subfractionized. In this tumor moderate pleomorphism was seen (fig. 12*B*).

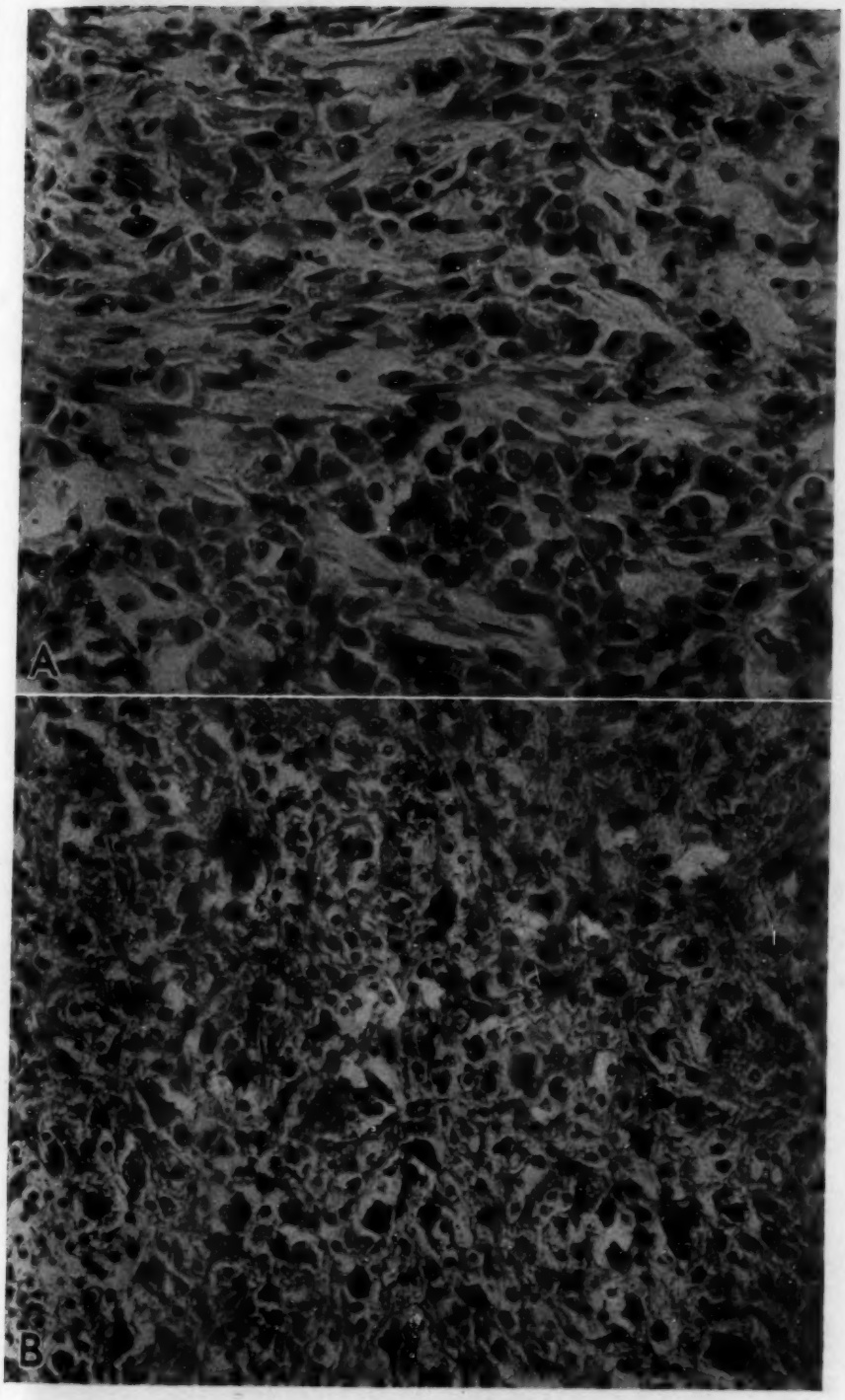

Fig. 12.—*A*, Walker tumor, an untreated control at six weeks; × 300. *B*, Walker tumor after twenty-one daily half minute treatments, subfractionized; × 300. Moderate pleomorphism may be seen.

COMMENT

The Time Factor: Bunsen-Roscoe Law.—The many studies that have been made in the field of radiobiology have been admirably reviewed in the monograph by Colwell,[4] the reviews by Packard [5] and the recent compendium in two volumes under the editorship of Duggar and others.[6] Expressed in the simplest terms, roentgen rays and radium induce atomic changes in the substance acted on. The rays displace electrons from their orbits, leaving the atoms in an altered electrical state. The changes that are observed in living matter following irradiation depend fundamentally on this changed electrical state. They include: alterations in the concentration of hydrogen ions and in the viscosity, permeability, surface tension and protoplasmic streaming; granulation, vacuolation or coagulation of the cytoplasm; changes in the mitochondria and Golgi bodies; a decrease in respiration and an increase in glycolysis as measured by the Warburg technic; changes in lipoids, enzymes, etc., and pyknosis of the nuclei and karyorrhexis, as well as alterations in the rhythm of mitosis and in the form of the mitotic figures.

An important concept that appears in the work of many radiobiologists is that with sublethal doses the protoplasmic changes are reversible. Protoplasm has the same power of recovery following irradiation as following other injuries. Attempts to arrive at doses just sufficient to exert a lethal effect have led, among other procedures, to the use of simple living forms as test objects. One type of result is represented in the outstanding work of Packard.[7] During a period of several years he used over 200,000 developing eggs of Drosophila in measuring the dose of radiation which when applied to a given batch of eggs, all about the same age, would kill 50 per cent (the proportion which, in all experiments of this type, is found to be the most significant). This dose is found to be remarkably constant, 190 r. It is constant enough so that the material can be used as a biologic measure of doses and checks with ionization chambers within narrow limits. The important aspect of these experiments is that within a limited range the intensity of the rays, i. e., the rate of application of the dose, makes no difference in the result. Packard emphasized that the developing eggs of Drosophila are practically independent of the time factor. Similar results were

4. Colwell, H.: The Method of Action of Radium and X-Rays on Living Tissues, London, Oxford University Press, 1935.

5. Packard, C., in Glasser, Otto: The Science of Radiology, Springfield, Ill., Charles C. Thomas, Publisher, 1933, p. 319; Quart. Rev. Biol. **6**:253, 1931.

6. Duggar, B. M., and others: Biological Effects of Radiation, New York, McGraw-Hill Book Company, 1936.

7. Packard, C.: Am. J. Roentgenol. **33**:317, 1935.

found by Faber[8] in tissue cultures. Sievert and Forssberg,[9] Laser[10] and a number of others working with similar biologic material have found the same results. Pack and Quimby[11] have listed experiments of this type in the literature.

The principle can be expressed in the form of a simple physical law: IT = K (I = intensity; T = time; K = a constant). This is the Bunsen-Roscoe law, first applied, in 1862, for the effect of light on the photographic plate. This apparently simple principle states that the biologic effect depends quantitatively on the total energy absorbed.

Schwartzschild's Law.—A large number of experiments have been performed similar to the aforementioned ones with greater variations in the intensity of the dose. These experiments have demonstrated that if a given dose is applied with weak intensity over a longer period of time (or if it is fractionated into several small doses), the total effect is *less* than if the same dose is applied with strong intensity in a short time. Apparently some of the energy is dissipated when it is spread over a longer period. Krönig and Friedrich[12] were among the first to discover this phenomenon of incomplete cumulation in the production of erythema. Holthusen[13] analyzed the problem. Canti[14] found the same thing true in tissue cultures. Liechti[15] and many others, too numerous to mention, have made similar observations. The effect comes daily to the attention of radiologists, who find that the skin can stand enormous amounts of radiation if it is applied a little at a time. The effect has been analyzed by Pack and Quimby,[11] Stenström and Mattick,[16] Kahlstorf,[17] Reisner,[18] Pape,[19] Miescher[20] and others, who have constructed tables and curves showing how much radiation the skin can tolerate in divided dose therapy. The large doses required to eradicate certain tumors by divided dose therapy possibly falls into the same category, although, as will be brought out later, an additional factor is perhaps involved.

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8. Faber, B.: Acta radiol., supp. 21, 1935, p. 1.
9. Sievert, R., and Forssberg, A.: Acta radiol. **12**:535, 1931.
10. Laser, H.: Strahlentherapie **38**:391, 1930.
11. Pack, G. T., and Quimby, E. H.: Am. J. Roentgenol. **28**:650, 1932.
12. Krönig, B., and Friedrich, W.: Physikalische und biologische Grundlagen der Strahlentherapie, Berlin, Urban & Schwarzenberg, 1918.
13. Holthusen, H.: Strahlentherapie **21**:275, 1926; **41**:435, 1931; **42**:881, 1932.
14. Canti, R. G.: Acta radiol. **10**:320, 1929.
15. Liechti, A.: Strahlentherapie **33**:1, 1929.
16. Stenström, W., and Mattick, W. L.: Am. J. Roentgenol. **15**:513, 1926.
17. Kahlstorf, A.: Strahlentherapie **38**:499, 1930.
18. Reisner, A.: Fortschr. a. d. Geb. d. Röntgenstrahlen **45**:293, 1932.
19. Pape, R.: Strahlentherapie **48**:73, 1933.
20. Miescher, G.: Acta radiol. **16**:25, 1935.

The foregoing observations also, apparently, can be expressed in the form of a simple law: $IT^n = K$ (I = intensity; T = time; n = the time factor, < 1; K = a constant). This is Schwartzschild's law, originally developed for the effect of very weak light intensities acting on a photographic plate for a long time; apparently, under such conditions a slight reversibility in the chemical change becomes manifest, which can be expressed accurately by the formula. In this formula the Bunsen-Roscoe law represents simply a special case in which the time factor, or exponent n, is equal to 1.

Again one has a simple physical principle which expresses the action of prolonged or fractionated irradiation on protoplasm in a quantitative manner in relation to the total energy absorbed. A logical explanation of this principle is that after each small dose a certain amount of reversible change takes place. With weak intensities the processes of recovery that characterize living matter come prominently into the picture. Canti [14] in his classic researches on tissue cultures demonstrated that there is a definite threshold of time and of intensity which must be reached before an effect can be observed. Therefore, each time a small dose is applied, the energy needed to reach this threshold is in a sense dissipated, and full cumulation does not take place. One finds much needless confusion in the literature on this subject. The principles outlined in the foregoing paragraphs, expressed by the Bunsen-Roscoe and Schwartzschild laws, appear to be logical explanations of that aspect of the rôle of the time factor which involves full cumulation with strong intensities (short time) and incomplete cumulation with weak intensities (long time), as observed in certain types of biologic material.

Exceptions to Bunsen-Roscoe and Schwartzschild Laws.—The aforementioned principles fail, however, to explain certain observations which involve a different aspect of the time factor. Essentially, these observations include a group of experiments in which weak intensities and divided doses, far from failing to cumulate, were found to have an augmented action. One of the earliest of these observations was that of Regaud.[21] He sterilized a ram's testis with radium by prolonged action at weak intensity, while a similar dose acting for a short time at high intensity left the testis intact except for local areas of necrosis. In the total energy expended the cases were similar, but in the final biologic result the case in which the weaker intensity was used showed enormous magnification of effect. Regaud and his collaborators have extended this work, and the observations have been confirmed by Schinz and Slotopolsky [22] and by others. Experimental studies on tumor trans-

21. Regaud, C.: Compt. rend. Soc. de biol. **86**:787, 1922.

22. Schinz, H. R., and Slotopolsky, B.: Acta radiol. **7**:365, 1926.

plants and other tissues have yielded similar results (Wood and Prime;[23] Zuppinger[24] and others). Clinical observations have accumulated also. Many a radioresistant tumor will disappear under divided dose therapy though it will not be affected by a massive dose of the same order of magnitude. Some of our own observations illustrate the occasional augmented action of divided doses. Six of eleven Flexner-Jobling carcinomas that disappeared under divided dose therapy were destroyed by less than 2,590 r, the lethal massive dose (Schwartzschild's law, $n>1$).

The Nucleus.—Although these examples of exceptions to the Bunsen-Roscoe and Schwartzschild laws are rather striking, they are no more so than the whole complex problem of the differential action of the rays between tumor and tumor bed. The results of our experiments lead us, in interpreting these problems, to emphasize the rôle of the nucleus. That the nucleus is the most radiosensitive part of the cell is an established fact. Cells that are actively dividing, as in epithelium, bone marrow, ovary and testis, are radiosensitive; stationary cells are radioresistant, as bone, muscle and nerve cells. The law of Bergonié and Tribondeau,[25] formulated in 1906 to express this fact, has stood the test of time. It is also well established that the time of mitosis is the period of greatest radiosensitivity of the cell. This explains why in an apparently homogeneous population of cells all are not equally sensitive at any given moment. During mitosis and shortly before, the cell is in a state of temporary instability with rapid shifts in its viscosity, and therefore susceptible to injury. There is disagreement as to which phase of mitosis is most sensitive, whether prophase, metaphase, or telephase. Strangeways and Hopwood[26] came to the conclusion that the premitotic period is also sensitive, perhaps the most sensitive, an opinion which is substantiated by Love[27] and also by Colwell.[4]

If fractional exposures could be given over such an interval of time as to catch all the cells of a tissue culture or a tumor during their most sensitive phase, namely, in mitosis, only a small amount of energy would be required to produce extensive destruction. Such an occurrence could easily explain the experiments described in a foregoing paragraph, in which Schwartzschild's law had the exponent $n>1$. This is the principle on which Coutard[28] bases his technic of protracted fractional

23. Wood, F. C., and Prime, F.: J. Cancer Research **4**:49, 1919.

24. Zuppinger, A.: Strahlentherapie **43**:701, 1932.

25. Bergonié, J., and Tribondeau, L.: Compt. rend. Acad. d. sc. **143**:983, 1906.

26. Strangeways, T. S. P., and Hopwood, F. L.: Proc. Roy. Soc., London, s.B **100**:283, 1926.

27. Love, W. H.: Nature, London **134**:252, 1934.

28. Coutard, H.: Am. J. Roentgenol. **28**:313, 1932; Lancet **2**:1, 1934; Strahlentherapie **37**:50, 1930.

dosage. By protracting each treatment at a weak intensity he hopes to expose more tumor cells during mitosis. Unfortunately this is only a theoretical possibility. The actual time of mitosis of a tumor cell is about one hour; and the life cycle, the time between mitoses, in the fastest growing tumor is several days and is much longer in slowly growing tumors. Therefore by irradiating even two hours per day, as Coutard does, actually only a small number of mitotic figures can be caught. If the premitotic phase is also sensitive, there is greater justification for protraction, because this phase may be fairly long in comparison with the life cycle of the cell. Even with this taken into consideration, the principle of protraction probably accounts for some but not for all of the phenomena observed in the destruction of a tumor by radiation.

Alteration of the Hereditary Material.—When a tumor is irradiated, the sensitive cells, after a period of latency, from a few days to one or two weeks, disintegrate in a characteristic manner by pyknosis and fragmentation of the nucleus (Ludford [3]). How do those cells that are refractory disintegrate in a tumor that is destroyed by radiation? We should like to emphasize that this is a question that has been much neglected. The answer that has been in vogue is that by simple fractioning, protractional fractioning or both these procedures all the cells are eventually caught in a sensitive state, namely, mitosis or premitosis. As already brought out, this is probably not correct.

Here and there in radiologic literature, however, one finds the opinion expressed that the remaining refractory cells are perhaps altered in some fundamental way so that they fail to survive. Abnormal cells such as giant cells are often seen in irradiated tumors and tissue cultures. These have been assumed to be nonviable, but no special study has been made of them. We believe that the evidence from our experiments justifies emphasizing these abnormal cells for separate consideration. In six of our experimental tumors we found that the surviving tumor cells were all transformed, under various forms of fractional doses, into a pure growth of abnormal hyperchromatic giant cells. These abnormal cells degenerated in a specific manner, by calcification of the nucleus. The observations indicated that surviving refractory cells are apparently transformed, through profound alterations in their hereditary material, into a race of abnormal cells which, although they proliferate for a time, finally die.

The effect of roentgen rays and radium on chromosomes in producing abnormal embryos and mutations is well known (see Duggar,[6] chapters 38-43). The fundamental work on this subject was by Muller,[29] who

29. Muller, H. J., in Glasser, Otto: The Science of Radiology, Springfield, Ill., Charles C. Thomas, Publisher, 1933, p. 305; Scient. Monthly **29**:481, 1929.

in his studies on the genetics of Drosophila demonstrated that mutations are produced by the direct action of the rays on the chromosomes and genes.

The key to the interpretation of our results seems to be the following fact: Muller,[29] Oliver,[30] Timoféef-Ressovsky and Zimmer [31] and others have demonstrated that the number of mutations produced by irradiation depends directly on the dose, irrespective of the interval of time. They have proved that even the weakest intensities and the smallest doses exert an effect on the hereditary material. "The effect is exactly proportional to the amount of energy absorbed, and is exactly cumulative over an indefinitely long period . . . even in successive generations . . ." (Muller). In other words, gene changes are irreversible changes. Evidence from another direction is recorded in the history of the pioneers of radiology. After long exposure to minute amounts of radiation the hereditary material of the epithelial cells of the skin becomes so altered that cancers develop.

The nucleus, therefore, is in a sense probably always sensitive. Even the smallest amounts of energy, applied a little at a time, cumulate fully. This is a very important conclusion from the standpoint of radiotherapy. If even small amounts of energy produce profound alterations in genes and chromosomes, there will result marked changes in a proliferating tissue such as a tumor. A small change in a gene results in a much magnified change in proliferating tissue derived from it. Can the genetic make-up of the refractory cells in a tumor be altered by repeated small doses so as to transform these cells into a race of abnormal cells which will finally die out? The evidence from our experiments demonstrates that this is possible. That these results are applicable to human cancer cannot be asserted at present.

The effect outlined would be desirable if it could be accomplished efficiently. Rapidly growing tumors, however, present a special problem, which is difficult and complex. In tumors treated by fractional doses there is a prolonged period of latency. A certain amount of time elapses before sufficient energy is accumulated to produce an effect. In fast growing tissues cumulation is poor (Holthusen [18]); i. e., Schwartzschild's law applies with $n<1$. During this period of latency a fast growing tumor will increase markedly in size and may metastasize. Furthermore, many experimenters, especially the botanists, have demonstrated that very small doses of radiation have a slightly stimulative or a pseudostimulative effect, increasing the rate of cell division. This has been much disputed, and the matter is by no means settled. But if true, it would render dangerous the exposure of a fast growing tumor to

30. Oliver, C. P.: Anat. Rec. **51**:110, 1931-1932.

31. Timoféef-Ressovsky, N. W., and Zimmer, K. G.: Strahlentherapie **53**:134, 1935.

repeated small doses such as are utilized in the fractional treatment. The problem in dealing with rapidly growing tumors is to destroy as many cells in as short a time as possible. Large doses are therefore required, i. e., the massive technic. Fortunately most of these tumors are radiosensitive, so that a moderate massive dose will destroy most of the tumor. A logical method of attack is as follows: Following a moderate massive dose, which will destroy much of the tumor (i. e., the sensitive cells), the remaining part of the tumor, which is refractory, can be treated with fractional doses to obtain its final destruction by the mechanism outlined, namely, alteration of the hereditary material of the tumor cells. Our experiments with the Jensen sarcoma, a fast growing radiosensitive tumor, point to this method as a logical one.

The Time Factor in the Histologic Changes in Irradiated Tumors.—Morphologic changes following irradiation are an expression of the chemical and physiologic changes that take place. Histologic studies have therefore always been important guides in determining lines of attack on the problem. The general changes in cells have been studied extensively and need no special comment here. The changes that take place in an irradiated tumor are exceedingly complex, and all the processes involved are by no means clear. The first to study irradiated tumors were Apolant, Dominici, Contamin, Clunet and later Lacassagne and Monod (Colwell;[4] Colwell and Russ[32]). The most important early work of this nature was done by Clunet and his co-workers. They studied both animal and human tumors and established that the tumor cells, after a period of latency, undergo disintegration and phagocytosis, and that later giant cells appear. In squamous cell carcinomas there is a tendency to keratinization. Finally a connective tissue scar remains, with an occasional cell containing a hyperchromatic nucleus. Lacassagne and Monod in 1922 studied animal and human carcinomas and reported the same findings. Their studies went into greater detail and covered alterations in the rhythm of mitosis and in the form of the mitotic figures, degenerative changes in the tumor cells and the appearance of giant forms. Donaldson and Canti[33] confirmed the aforementioned findings in fifty cases of carcinoma of the human uterine cervix in which treatment was by radium. Ludford[8] in 1932 made an extensive histologic study of irradiated mouse tumors, probably the best study in this field. In addition to cytoplasmic changes, he found characteristic degenerative changes in the nucleus. Following a variable but short period of latency a characteristic type of pyknosis and fragmentation occurs. The chromatin runs together into large granules, often asso-

32. Colwell, H., and Russ, S.: Radium, X-Rays, and the Living Cell, London, G. Bell & Sons, 1924.

33. Donaldson, M., and Canti, R. G.: Brit. M. J. **2**:12, 1923.

ciated with vacuolation of the nucleus and thickening of the nuclear membrane. Then the nucleus breaks up into fragments, which become scattered through the cytoplasm. Such degenerative changes are also seen in tissue cultures and similar material.

When a sensitive cell is irradiated, death of the cell does not follow the changes in the cytoplasm, which are often reversible, but follows changes in the nucleus, on the integrity of which the cell depends for its existence. In an irradiated tumor the bulk of the cells, i. e., the most sensitive cells, probably disintegrate in the aforementioned manner. In the present experiments this type of primary degenerative change was seen especially in the tumors treated with single massive doses.

In addition to these degenerative changes, abnormal forms and giant cells have been seen after irradiation in almost every type of material studied. Although they have attracted much interest, their significance has been lost in the complexities of the problem. Strangeways, Colwell and Holthusen called attention to their puzzling character. We believe that our experimental material demonstrates that these giant cells deserve separate consideration.

No histologic studies have heretofore been made of the effect of divided doses. In the present experiments certain characteristic nuclear changes were seen in the tumors that were treated by divided doses, which were different from the primary degenerative changes described. These changes consisted of a gradual transformation of all the refractory tumor cells into hyperchromatic giant cells. These giant cells disintegrated, not by the pyknosis and fragmentation described, but in a specific manner, i. e., by calcification of the nucleus. In the tumors treated by divided doses many cells (the most sensitive) are seen to undergo the primary degenerative change. But the prominent part of the picture concerns the gradual transformation of the remaining refractory cells, by alteration of the hereditary material, into giant cells which calcify.

An interpretation of these findings seems to depend, as already brought out, on the researches of the geneticists. They have shown (Muller and others) that mutations following irradiation may result either from unequal distribution of chromosomes in daughter cells (nondisjunction) or from alterations in the chromosomes themselves. The first type of mutation, due to nondisjunction, results in abnormalities that appear immediately. This explains the appearance of giant cells in almost any proliferating tissue or tumor soon after a single exposure. The second type of mutation, which the geneticists have shown to develop from changes in the structure of the chromosomes and genes, is usually recessive and does not appear until several generations later. A certain amount of time is required before the morphologic result of this altera-

tion in chromosomes becomes apparent. In irradiated tissue cultures many cells can be seen to divide normally several times before abnormal forms appear. In irradiated tumors it is only in the later stages that giant cells develop in abundance.

Thus, two types of giant cells are probably produced by irradiation. The first type develops from sensitive cells as a result of nondisjunction of chromosomes. The second type is derived from refractory cells. It develops late, results from alterations in the structure of the chromosomes and genes and degenerates in a specific manner, by calcification of the nucleus.

The controversial subject of the rôle of the tumor bed or stroma cannot be gone into here. All the evidence in these experiments speaks for a primary action of the rays on the tumor cells, not on the stroma. We found no changes in the blood vessels, and all the mesenchymal reactive processes were secondary to degenerative changes in the tumor cells. With regard to the process of calcification seen in our tumors, only one similar observation can be found. Kluge and Zwerg [34] found a 400 per cent increase in the calcium content of irradiated Jensen rat sarcomas, as determined by chemical analysis of the gross tumors. They made no histologic studies.

The Time Factor in Radiotherapy.—The concept of the rôle of the time factor in the treatment of malignant tumors has changed repeatedly in the course of the development of radiotherapy. In the early years fractional applications predominated. One can hardly dignify these early attempts with the term "fractional method"; they consisted simply in taking small hesitant steps, since the early radiologists were entirely in the dark without dosimeters and without any conception of what the tissues could stand. During the war, under emergency conditions, several German radiologists (Krönig and Friedrich and others) gave as heavy an application within as short a period of time as the patient could tolerate, even with multiple portals of entry for deep tumors. Kingery [35] in 1920 and Pfahler [36] in 1926 introduced the saturation technic whereby an initial fairly large dose was followed by repeated small doses with the purpose of keeping the tissues saturated with as much radiation energy as they could tolerate.

More recently Coutard [28] introduced the protracted fractional method. The theoretical principle underlying this method may be explained as follows: By using very penetrating rays of low intensity over a long period of time, Coutard hopes to irradiate all the tumor cells during

34. Kluge, L., and Zwerg, H. G.: Strahlentherapie **46**:293, 1933.

35. Kingery, L. B.: Arch. Dermat. & Syph. **1**:423, 1920.

36. Pfahler, G. E.: Brit. J. Radiol. **31**:45, 1926; Am. J. Roentgenol. **20**:233, 1928.

their period of greatest sensitivity, namely, in mitosis. Coutard's method has varied quite a little, because he experimented with different technics until he found the one most suitable. In general, the treatment factors are such that the hourly intensity is from 200 to 300 r. By irradiating for from two to three hours daily for from ten to forty days, total doses of 5,000 to 16,000 r are given, the optimum being about 8,000 r.

As a guide to the correct dosage Coutard attempts to produce what he calls "radio-epidermitis" and "radio-epithelitis." The underlying principle for this goes back to Regaud's experiments on testes. By destroying the most sensitive cells, the spermatogonia, Regaud was able to sterilize the testis without the necessity of using larger doses for the more differentiated cells. The latter go on to the formation of spermatozoa, which disappear; but no new spermatozoa can be formed because the primary germ cells are destroyed. Similarly, Regaud postulated a *souche cellulaire,* or mother cell layer, at the periphery of alveoli of tumor cells, best illustrated in hornifying squamous cell carcinoma. According to Regaud, it is sufficient to destroy this peripheral undifferentiated layer. The hornifying cells, or *lignes laterales,* he claimed, are not capable of independent proliferation. Coutard, therefore, in irradiating tumors of the oral cavity tries to give a dosage sufficient to injure the basal cell layer of the adjacent normal skin, which he considers to have about the same radiosensitivity as the *souche cellulaire* of hornifying squamous cell carcinoma. This furnishes a guide to the dosage effective for the tumor. The epithelium of the mucous membranes in the vicinity of the tumor undergoes the same changes. The denuded areas of the skin and mucous membrane soon heal over from the peripheral intact skin.

It can be seen that Coutard's method combines several principles which had been established before he began their use. The technic of fractional dosage, of multiple portals of entry (maximalizing dosage) and of heavy filtration to get more penetrating rays had been in daily use. The principle of saturation was developed by Pfahler. Coutard combined all these principles. To compare the effect of his method on malignant tumors with the effects of other methods is difficult. Clinical results have been the only guide, and they have been variable. In our experiments no significant differences, either gross or microscopic, were found in the results of the protracted fractional treatment as compared with those of the simple fractioning treatment.

There are several important criticisms of Coutard's method. One is that it is theoretically impossible to catch more than a small number of tumor cells during mitosis. A second is that the concept of the *souche cellulaire* and the *lignes laterales* is certainly not valid. The differentiated cells in hornifying squamous cell carcinoma are capable of independent proliferation. A third critcism is that the method is not suitable

for fast growing tumors, which require massive doses. Other criticisms concern the expense and the inconvenience. The life of an x-ray tube being only a few hundred hours, only a comparatively small number of patients can be treated per tube. The time of physicians, nurses and attendants must be considered. The inconvenience to patients in coming twice a day for thirty or forty days and remaining for long periods in uncomfortable positions during the treatments makes it difficult to gain their cooperation.

The effects on the adjacent normal tissues and on the patient in general of the divided dose methods have not received a great deal of attention. The general subject of skin tolerance has been most studied. As high a dose as 270 per cent can produce the same erythema as a 100 per cent dose if the former is spread over twenty-seven days. A 320 per cent dose will produce only mild dermatitis when spread over sixteen days. Kahlstorf [17] studied the protracted fractional method in rabbit's ears. Four epilation doses applied by this method produced no epilation in from fourteen to twenty weeks. From five to six erythema doses applied to human skin by this method resulted in only minor degenerations of cells, as shown by biopsy. Czunft and Gaál [37] found no differences between Coutard's method and simple fractioning in biopsies of human skin. Miescher [20] recently reported that the threshold for late effects (baldness, atrophy and hyperkeratosis after from one to four years) is lower than for immediate effects.

Little can be found in the literature regarding other tissues. Changes in the blood with Coutard's method were studied by Gloor and Zuppinger [38] and by Zwerg.[39] The former studied eleven patients and found that the changes, although essentially of the same kind as with other methods, were slight in comparison with the large dose. Zwerg found the same thing true in both patients and experimental animals. With the Coutard method Zwerg found no damage in the lungs, heart and kidneys of chicks, whereas massive doses damaged such tissues.

SUMMARY

The result of greatest interest in these experiments was the demonstration of two different types of degenerative changes in irradiated tumor cells. One of these is well known; it is the primary degeneration or necrosis of the tumor cell which follows soon after the absorption of sufficient radiation energy by the cell. It consists chiefly in a characteristic pyknosis of the nucleus and karyorrhexis. This is the process by which sensitive cells degenerate, i. e., those in mitotis or in the premitotic phase.

37. Czunft, W., and Gaál, A.: Strahlentherapie **54**:616, 1935.
38. Gloor, W., and Zuppinger, A.: Strahlentherapie **40**:438, 1931.
39. Zwerg, H. G.: Strahlentherapie **43**:201, 1932.

The second type of degenerative change which these experiments demonstrated is that which occurs in refractory cells following irradiation by divided dose methods. It consists of a profound alteration of the hereditary material of these cells (chromosomes and genes) so that they are transformed into abnormal forms which fail to survive and which degenerate in a specific manner. The refractory cells in our tumors became transformed, under various forms of divided dose therapy, into large giant cells with hyperchromatic nuclei, which eventually calcified. Several of our Flexner-Jobling carcinomas turned into practically pure cultures of such cells.

From the standpoint of radiotherapy of tumors, this second type of degenerative change which develops in refractory cells is of great importance for the following reason: The effect of small divided doses is fully cumulative on the nucleus in producing this result, as the geneticists have proved. In radiotherapy, this suggests the possibility of destroying a tumor by divided dose methods, when the lethal massive dose for the tumor is injurious to the host. The tumor bed cumulates divided doses of radiation poorly (Schwartzschild's law, $n<1$). Tumor cells also cumulate divided doses of radiation poorly as far as primary degenerative effects are concerned. However, comparatively small changes in the genes and chromosomes of the cells of a tumor become greatly magnified, because it is a proliferating tissue.

Protraction of the individual divided dose, in these experiments, produced no significantly different effect, as compared with simple fractioning. From a theoretical standpoint there is no sound basis for protraction (except for the benefits on the host of weak intensity) because it is a practical impossibility to irradiate a large number of cells during mitosis unless the irradiation is continuous. Good results obtained by Coutard's method are probably due to fractioning alone. It seems logical to believe that divided dose methods act not by catching more cells during a sensitive phase (mitosis) but by altering the hereditary mechanism of the cells in such a manner that subsequent generations are abnormal and fail to survive.

The most efficient method of treatment in these experiments was the saturation method. This was especially evident in fast growing tumors (Jensen sarcoma). This method seems to represent a logical balance between massive doses and divided doses. The nuclear changes discussed take time to develop. During this period of latency the increase in the size of a fast growing tumor may be dangerous. An initial treatment with a moderate massive dose destroys the sensitive cells; in a fast growing tumor there are many cells in mitosis and premitosis, so that an appreciable portion of such a tumor is destroyed in this way. The remaining refractory cells can be destroyed at a more leisurely rate with subsequent divided doses.

CONCLUSIONS

Following irradiation sensitive tumor cells undergo primary degenerative changes which are of the nature of simple necrosis.

Refractory tumor cells become altered in their chromosomal structure by irradiation, an alteration which results in the development of abnormal forms (giant cells, in the tumors described in this paper).

These abnormal forms developing as a result of the action of the rays on the nuclei fail to survive. They degenerate in a specific manner (in the tumors described in this paper, by calcification of the nuclei).

The effect of small divided doses of radiation is fully cumulative in producing changes in the chromosomes and genes of refractory cells.

Because of this full cumulative effect on their nuclei, divided dose methods offer the possibility of destroying a tumor by transforming its cells into abnormal forms, when the lethal massive dose for the tumor is injurious to the host.

Protracting the individual divided dose is probably of no value. In the experiments described in this paper no significant gross or microscopic differences in effects were found between the fractional and the protracted fractional treatments.

Fast growing tumors may increase in size to a dangerous extent before the effect of divided doses becomes manifest. For such tumors the saturation technic is a logical choice. An initial moderate massive dose destroys the sensitive cells. Subsequent divided doses destroy the refractory cells by the mechanism of altering their hereditary structure. In the experiments reported such a saturation method was found to be most efficient.

NASOPALATINE DUCT STRUCTURES AND PECULIAR BONY PATTERN OBSERVED IN ANTERIOR MAXILLARY REGION

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Cysts in the anterior maxillary region have attracted the attention of both anatomists and clinicians. The anatomist Meyer [1] first noted these structures in 1908. Later, in a more complete study [2] based on dissected material, he obtained data concerning the origin, frequency and anatomic relations of these cystic structures.

Case histories, roentgenograms and in some instances photomicrographs of the removed cysts were included in the more complete clinical studies of Schroff,[3] Neuwirt and Parma [4] and Bauer.[5] Stafne, Austin and Gardner [6] determined the incidence of these structures, which could be diagnosed by means of visual and roentgenologic examinations of the implicated region. Although numerous case reports [7] dealing with these cysts have appeared, histologic studies [8] have been few. Thoma [9] differentiated cysts arising in the palatine papilla from those occurring in the anterior palatine canal (incisive canal).

Specimens obtained at operation have usually been used for these studies. However, when the cysts have become clinically recognizable, definite information as to their exact anatomic site or possible origin is not always possible. In the operative procedures, as a rule only the cyst lining or the exposed bony wall and part of the lining are

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From the Department of Pathology, Yale University School of Medicine. A contribution from the Dental Study Unit.

1. Meyer, A. W.: J. Anat. & Physiol. **48**:118, 1914.
2. Meyer, A. W.: J. Am. Dent. A. **18**:1851, 1931.
3. Schroff, Joseph: J. Dent. Research **10**:739, 1930; Dent. Items of Interest **51**:107, 1929.
4. Neuwirt, F., and Parma, C.: Ztschr. f. Stomatol. **30**:1195, 1932.
5. Bauer, W.: Ztschr. f. Stomatol. **28**:481, 1930.
6. Stafne, E. C.; Austin, L. T., and Gardner, B. S.: J. Am. Dent. A. **28**:801, 1936.
7. Rushton, M. A.: Brit. Dent. J. **51**:109, 1930. Cohen, M., and Levine, J.: Internat. J. Orthodontia **22**:526, 1936. Blum, Theodore: J. Am. Dent. A. **16**: 649, 1929. Wild, H. D.: Dent. Items of Interest **52**:63, 1930.
8. Kronfeld, R.: Histopathology of the Teeth and Their Surrounding Structures, Philadelphia, Lea & Febiger, 1933, p. 406. Thoma, K. H.: Clinical Pathology of the Jaws, Springfield, Ill., Charles C. Thomas, Publisher, 1934, p. 266.
9. Thoma, K. H.: Internat. J. Orthodontia **22**:521, 1936.

removed, with the result that the exact relationship of the cystic cavity and associated glandular structures to the surrounding alveolar bone cannot be studied adequately.

Cysts in the median anterior maxillary region are believed to arise from remnants of the nasopalatine duct or epithelial rests enclaved during fusion of the bony processes in the course of formation of the anterior portion of the hard palate.[10] In dogs, rabbits [11] and monkeys the nasopalatine duct persists as a demonstrable communication between the oral and nasal cavities. A similar communication occurs in the early fetal life of man and in the new-born infant.[12] According to Peter,[13] the ducts usually become obliterated and are represented by cords of epithelial cells continuous at one extremity with the nasal epithelium and at the other with the palatal epithelium. Persistence of small portions of the nasopalatine duct may explain the presence of small cystic structures and epithelial cells in the maxilla dorsal to the upper incisor teeth.

Rawengal's [14] embryologic studies showed that there are three sets of epithelial tissues in this region: (1) the nasopalatine duct or its rests, (2) a middle epithelial cord in the incisive canal and (3) epithelial rests in the most posterior portion of the palatine papilla. According to this investigator, the epithelial tissues aside from the nasopalatine duct rests disappear in most cases in the first year of life. Except for these embryologic studies, little is known of the possible pathogenesis of these cysts or of the frequency with which they may occur without producing clinical symptoms.

It is hoped that the anatomic findings presented in this report will contribute to the knowledge of the causes, pathogenesis and pathologic changes of these structures and in addition stimulate further investigation of the peculiar bony formation commonly found in the anterior maxillary region.

MATERIAL

The human necropsy material used in this study was secured for a bacteriologic, roentgenologic and histologic study of tooth apexes. The finding of numerous small cysts and of the peculiar bony pattern in the anterior maxillary region was not anticipated. These structures were first believed to be remnants of Jacobson's organ; however, their anatomic relations and further histologic study aided by special neural strains definitely excluded this possibility.

When the frequent occurrence of these anterior median cystlike structures was realized, the remaining specimens were divided and sectioned so that serial preparations were available throughout this region. They included the alveolar bone surrounding the maxillary first incisors and usually the floor of the nasal fossa.

10. Grohs, Richard: J. Dent. Research **14**:187, 1934.
11. Wermuth, Hans: Deutsche Monatschr. f. Zahnh. **45**:203 (March 1) 1927.
12. Noyes, H. J.: J. Dent. Research **15**:155, 1935.
13. Peter, Karl: Arch. f. mikr. Anat. **97**:523, 1923.
14. Rawengal, G.: Arch. f. mikr. Anat. **97**:507, 1923.

The specimens were fixed in solution of formaldehyde U. S. P. after roentgenograms were taken and periapical cultures made by a modification of the root amputation technic. A double celloidin plus paraffin embedding technic was used.[15] Serial sections of 12 microns thickness were cut in a buccolingual plane from the distal surface of one incisor to the corresponding surface of the adjacent teeth. These sections included the tooth and surrounding alveolar bone. They were stained as a routine with hematoxylin and alcoholic eosin, but Masson's[16] trichrome staining technic was also used.

RESULTS

Thirty-five anterior maxillary specimens were available for study. A peculiar bony pattern was observed in this region in twenty-three instances, and microscopic or macroscopic cystic structures or rudimentary nasopalatine ducts were seen in twenty-one maxillae. The bony pattern and small cystlike structures (persisting portions of the nasopalatine duct) were noted in each of the last fifteen necropsies studied in which serial sections were made. Failure to observe these structures in the earlier cases was due probably to the methods of dividing and sectioning the specimens.

The ages of the subjects from whom material was obtained ranged from 32 to 73 years. The pathologic entities, as indicated by the primary anatomic diagnosis, included generalized vascular disease, five cases, carcinoma, four cases, rheumatic heart disease and subacute bacterial endocarditis, three cases, pneumonia, three cases, meningitis, two cases, and Paget's disease, accidental death and postoperative death, one case each.

Gross examination of this region had revealed no abnormalities, such as palatal swellings or diastasis of the upper central incisors. There was great variation in the roentgenologic findings in this region, and evidence of a cystic lesion was thought to be present in two cases.

There was macroscopic evidence of anterior median maxillary cysts in approximately 1.5 per cent of the dissected specimens which Meyer examined. Definite cysts were observed in 33 per cent of the present series, and rudimentary nasopalatine duct structures were seen in an additional 30 per cent. The minute study of this region made possible by serial histologic sections revealed a high incidence of cystic structures unrecognizable by the usual methods of examination. In many instances these cystic cavities were large enough to be demonstrable grossly if the specimen had been cut in the proper sagittal plane (fig. 1).

The incisive canal occupied a comparatively large area in the alveolar bone and was filled with loose fibrous connective tissue, numerous mucoserous glandular acinae and persisting portions of the nasopalatine duct

15. Burket, L. W.: A Post-Mortem Study of the Gross, Periapical Bacteriological and Roentgenological Findings of 419 Human Teeth, Thesis, Yale University School of Medicine, 1936.

16. Foot, N. C.: Stain Technol. **8**:101, 1933.

or cystic derivatives from this structure. The duct was usually lined with ciliated columnar epithelium (fig. 2*A*). In addition, numerous nerve trunks and a rich vascular network were present.

The oral termination of these cystic aberrations of the nasopalatine duct was always slightly lateral and lingual to the small ovoid mass of hyaline cartilage present in the midline in the region of the anterior palatine papilla (fig. 2*B*). The oral extremity was represented by a duct of varying size or by a plug of epithelial cells continuous with the mucous membrane of the palate. The duct was lined with ciliated columnar epithelium to within from 3 to 4 mm. of the palatal opening, where it was replaced by a transitional and then a typical squamous type of epithelium, often with a well cornified surface. In ten cases the oral opening was patent.

The nasal extremity, corresponding to the depressions in the floor of the nasal fossa marking the opening of the nasopalatine duct, was

Fig. 1.—Actual size of cyst; hematoxylin and eosin. The relation of the cyst in the incisal canal to the tooth, palatal gingiva and nasal fossa is shown. Irregular bony trabeculae are present in the palatal area near the nasal fossa.

more lateral than the oral termination. A patent nasal opening was observed in eight cases.

Many of the cystic cavities appeared empty, and it is unlikely that any material was lost during the preparation of the sections, as a double celloidin embedding procedure was used. The cystic areas were filled occasionally with a dense pink-staining homogeneous material. In these instances no patent opening to the oral cavity was found, the oral termination being represented by a mass of epithelial cells lingual to the cartilage pad and continuous with the mucous membrane of the mouth. In four of the cysts evidence of infection was present, which consisted of a dense polymorphonuclear leukocytic accumulation within the cavity with a marked cellular reaction in the connective tissue adjacent to the epithelial lining (fig. 2*C*). A minimal round cell accumulation was present in the region of the mucoserous glandular acinae. When

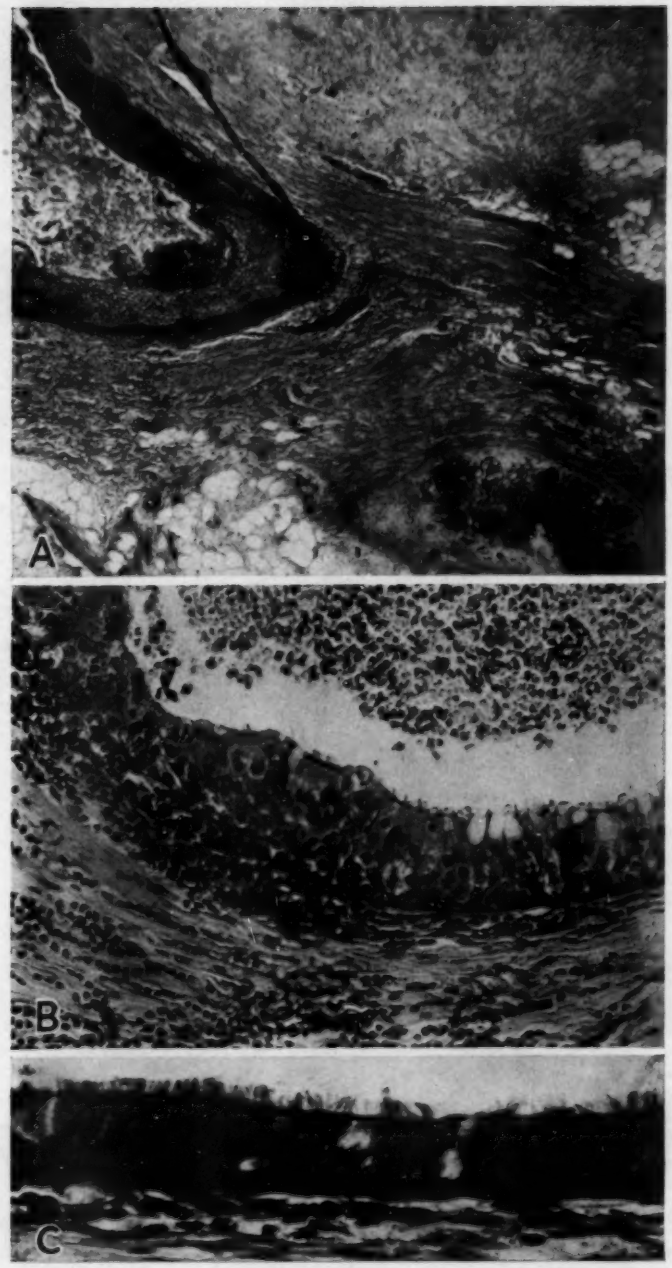

Fig. 2.—*A*, oral opening of persisting nasopalatine duct and its relation to the hyaline cartilage pad which is present in the region of the palatine papilla; × 35; Masson stain. *B*, infected cyst with contained exudate and a small round cell infiltration in the surrounding connective tissue; metaplasia of ciliated epithelium to a transitional form; × 115; hematoxylin and eosin. *C*, ciliated epithelium lining the nasopalatine duct and derived cystic structures; × 230; Masson stain.

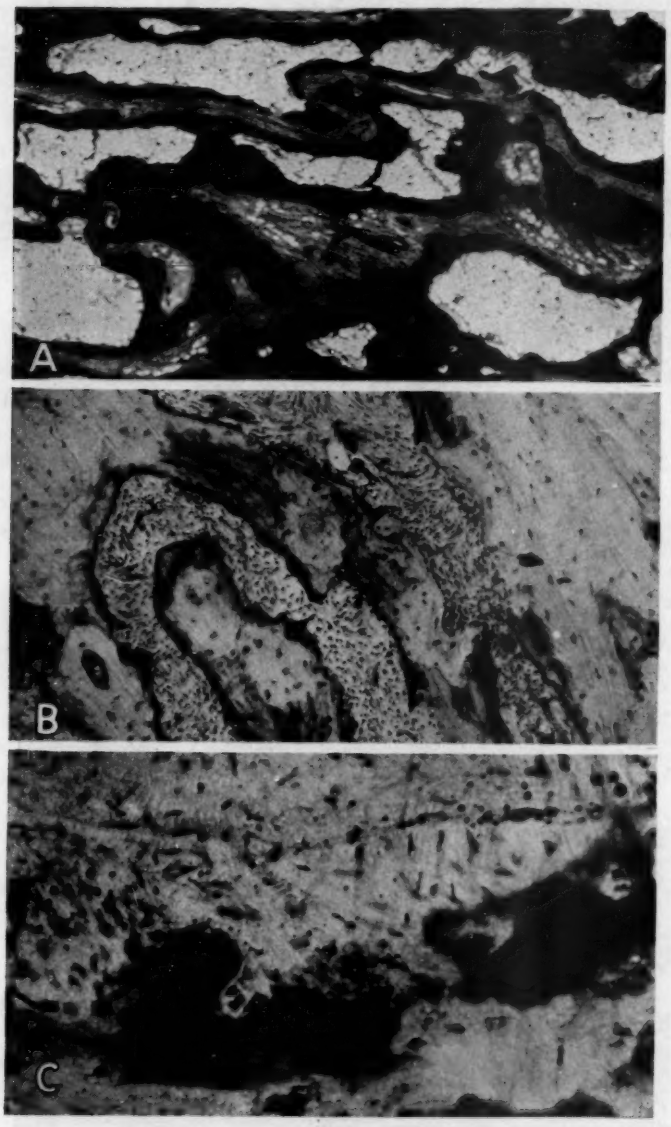

Fig. 3.—*A*, unusual bony pattern composed of irregular bony trabeculae with borders staining deep blue and cellular intertrabecular tissue; × 35; hematoxylin and eosin. *B*, anterior maxillary bony pattern resembling osteitis fibrosa, localized, with fibrous intertrabecular tissue; × 35; hematoxylin and eosin. *C*, small calcified granules in the intertrabecular connective tissue of the anterior maxillary region; × 115; hematoxylin and eosin.

the cystic cavities were filled with the homogeneous pink-staining material or with inflammatory exudate, the usual ciliated columnar epithelium was replaced frequently by a squamous type of varying thickness. Epithelial rests other than those related to the nasopalatine duct were not observed in this region.

Unusual and peculiar osseous structure was observed in the region of the nasal fossa. The histologic appearance was similar to that which Thoma described as occurring in osteitis fibrosa. The bony trabeculae were wider, more numerous and more irregular than elsewhere. Many of the lamellae had a border staining deep blue and showed evidence of both bone building and bone absorption (fig. 3 *A*). The spaces between the trabeculae were filled with dense fibrous connective tissue and in some instances small calcific masses, which were arranged parallel to the numerous small blood vessels present in the intertrabecular tissue (fig. 3 *B* and *C*).

COMMENT

This study correlates certain roentgenologic findings commonly observed in the anterior maxilla with large incisive canals containing cystic and glandular structures which were seen in the serial microscopic preparations. The association of these cysts with certain roentgenologic areas in the anterior median maxilla has been stressed only in recent years.[17] The high incidence of these cystic structures in the incisive canal and the correspondingly high incidence of a large or unusual palatine foramen as observed in the roentgenograms have not been associated previously. Meyer stated that "it is evident that small cysts could easily escape detection in medial sagittal sections made with a large carpenter's saw by students not fully aware of what might be expected and not skilled in the use of the saw." "This applies especially to cysts such as suggested in the roentgenograms from children under 14. Such small specimens would probably be almost or indeed wholly destroyed."

The significance of these cystic structures is difficult to evaluate. Only a few give rise to clinical symptoms because of their expanding growth or because of infection. No history relative to the four infected cysts could be obtained in the hospital records. An infected cyst might serve as a focus of infection. The unusual vascularity observed in the region of the cysts might explain the postoperative hemorrhage which occasionally follows operative removal of the lesion.[3]

The microscopic study of these structures in situ permitted a more accurate interpretation of their anatomic relations and probable etiology. The universal appearance of ciliated columnar epithelium in this region indicates a primary connection with the nasal cavity and a probable derivation from the rudimentary nasopalatine duct. Adjacent to the

17. Goodman, Moses: Radiology **26**:157, 1936.

persisting portions of these cystic structures and especially numerous near the nasal floor are many seromucous acinae. The ducts from these acinae open into the persisting portions of the nasopalatine duct or its derived cystic structures. It is possible that the secretory activity of these small glands prevents more frequent infection of this narrow duct. On the other hand, when terminal occlusion or blockage occurs, they may play a part in the production of an expanding type of growth. However, the ciliated columnar epithelium lining this nasopalatine duct contains numerous goblet cells which might themselves produce cystic change without activity of the adjacent glands.

The openings of the nasopalatine duct were always slightly lateral and lingual to the center of the cartilage pad observed constantly in the midline just lingual to the dental arch. If portions of the nasopalatine duct in this region undergo cystic change, a cyst of the palatine papilla might result; if an intraosseous portion of the nasopalatine duct undergoes a similar change, a median anterior maxillary cyst might result.

The bony pattern observed in the anterior maxilla near the floor of the nasal fossa has not been described in persons without known osseous lesions. Similar findings were not observed in relation to the maxillary sinus or nasal fossa distally in these same necropsies. The appearance of the bone is similar to the mosaic patterning seen in Paget's disease or that described by Thoma in localized osteitis fibrosa. However, in the case of Paget's disease included in this study the bony markings in this region were not as definite as in many of the other cases. The altered arrangement and increase in number of the bony trabeculae were striking. In many instances their direction and arrangement suggest a response to some functional stress. In other specimens the intertrabecular spaces were filled with dense fibrous connective tissue, which contained, in many instances, a diffuse deposition of calcific masses.

SUMMARY

Portions of the rudimentary nasopalatine duct were present in twenty-one of thirty-five specimens studied. They were present in each of the fifteen adult human specimens which were examined by the method described.

Portions of the nasopalatine duct are present and may give rise to the cystic structures found in the anterior palatine papilla and median anterior maxillary region.

A peculiar bony pattern was observed in the anterior maxilla near the nasal fossa. The cause and significance of this anatomic change is not known.

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ANATOMIC AND BACTERIOLOGIC FINDINGS IN INFECTIONS WITH SPECIFIC TYPES OF PNEUMOCOCCI, INCLUDING TYPES I TO XXXII

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For the past few years, since specific serums have become available for the classification of practically all pneumococci, including those previously placed in group IV,[1] there has been a continuous effort made at the Boston City Hospital to evaluate the relationship of the various types of pneumococci to disease. One of us (Dr. Finland[2]) has recently presented a brief résumé of 3,682 cases in which, between November 1929 and June 30, 1936, specific types of pneumococci were obtained and classified with the aid of these newer serums. This series included 764 cases in which autopsies were made. It is of interest to present the more important anatomic and bacteriologic findings in this group of cases. It is hoped thus to shed further light on the relative significance of the various types of pneumococci in disease, on the end-results of the infections which they produce and on the relationship of certain factors in the host to infections with these different types.

CASES INVESTIGATED AND SOURCES OF PNEUMOCOCCI

The present study includes every case with autopsy which occurred during the period mentioned in which a specific type of pneumococci was identified in materials obtained from any source during life or at autopsy. The clinical, pathologic and bacteriologic data that were obtained were all utilized. Dr. Frederick Parker Jr. and Dr. Robert N. Nye of the Mallory Institute of Pathology permitted us to use the autopsy protocols and the bacteriologic data.

This study was aided in part by a grant given in memory of Francis Weld Peabody by the Ella Sachs Plotz Foundation.

From the Thorndike Memorial Laboratory, Second and Fourth Medical Services (Harvard), Boston City Hospital, and the Department of Medicine, Harvard University Medical School.

1. Cooper, G.; Edwards, M., and Rosenstein, C.: J. Exper. Med. **49**:461, 1929. Cooper, G.; Rosenstein, C.; Walter, A., and Peizer, L.: ibid. **55**:531, 1932.

2. Finland, M.: Ann. Int. Med. **10**:1531, 1937.

Prior to June 1932, typing serums were available only for type I to XX. During this period there occurred among the cases coming to autopsy 12 with pneumococci that failed to agglutinate in the available serums. These cases are included in the present study. Since June 1932, serums for types I to XXXII have been used. We obtained through Dr. William H. Park and the late Miss Georgia Cooper of the Laboratories of the New York City Department of Health our supply of the serums for all the newer types. Pneumococci were obtained during life: (1) from sputum, and occasionally from cultures of pharyngeal swabs, by inoculation of mice, (2) from blood cultures and (3) from cultures of exudates. At autopsy cultures were made: (1) from cardiac blood, (2) from consolidated portions of the lung and (3) from exudates. All pneumococci were isolated in pure culture and typed. Other significant organisms were also identified.

EXPLANATION OF THE TABLES

The designation "lobar pneumonia" is used to describe inflammatory consolidation of the lung which, within a given lobe, is uniform in character and contiguous in distribution and which involves a considerable portion of that lobe. The term "atypical pneumonia"[3] is used instead of the more common term "bronchopneumonia" to describe patchy or irregular inflammatory consolidation. When both types of involvement were noted in different lobes, the pneumonia was classified as mixed (lobar and atypical).

For the sake of brevity we have listed separately, in the tables concerned with pneumonia, only the types of pneumococci represented by 10 or more cases each. (The 9 cases of atypical pneumonia due to type IV were also listed because that type is noted under lobar pneumonia.) The remaining types, which are each represented by less than 10 cases, were listed together. The 31 cases of lobar pneumonia so grouped include the following types (Roman numerals) and their respective numbers of cases (Arabic numerals): VI, 5; IX, 4; X, 4; XI, 1; XII, 3; XIII, 1; XIV, 6; XVIII, 4; XIX, 1; XXI, 1, and XXII, 1. The 76 cases of atypical pneumonia that are grouped together include: IX, 6; XI, 8; XII, 9; XIII, 4; XIV, 8; XVI, 6; XVII, 7; XIX, 3; XXI, 2; XXII, 5; XXIII, 3; XXIV, 1; XXVII, 1; XXVIII, 1; XXIX, 2, and no agglutination in types I to XX, 10. The 31 grouped cases of mixed (lobar and atypical) pneumonia include: II, 2; IV, 2; V, 7; VII, 5; VIII, 3; X, 2; XII, 1; XIV, 5, and XVII, XVIII, XIX and XX, 1 each.

The 44 cases in which serum was used in treatment were listed with their respective types and also grouped together under the heading "serum-treated." They include the following: lobar pneumonia—I, 21; II, 14, and V, 2; atypical pneumonia—VII, 1; mixed (lobar and atypical) pneumonia—I, 4; V, 1, and VII, 1. All of the patients received the homologous type-specific concentrated antibody intravenously in varying amounts and at different stages of their illness.

In the tables the numbers represent cases, and the use of percentage is avoided. In tables 1 to 4, the percentage of cases in each category may be obtained by comparing with the total noted above each column.

RÉSUMÉ OF THE CASES WITH PNEUMONIA

In 684 cases there was present at autopsy pneumonia that could be attributed to the pneumococci. These included 339 cases in which

3. Cole, R. I.: Acute Pulmonary Infections, in DeLamar Lectures, 1927-1928, Baltimore, Williams & Wilkins Company, 1928.

typical lobar pneumonia was observed, 290 cases in which atypical consolidation was found and 55 cases in which mixed pneumonia was present, i. e., lobar pneumonia in one or more lobes and atypical pneumonia in other parts of the lung. In a considerable proportion of the cases, mainly among those in which atypical pneumonia was noted, the pulmonary lesion was first noted at autopsy and adequate clinical and bacteriologic data relating to the pneumonia were not obtained during life. In many of these cases, the pneumonia served only as a terminal event or as a factor contributing to death from other causes.

The more important features of these cases are listed in tables 1 to 4 according to the character of the pulmonary lesion and the type of pneumococcus recovered. Some of these findings may be summarized and commented on briefly.

Distribution of Types According to the Character of the Pulmonary Lesion.—The commonest types among the cases of lobar pneumonia were types I, III and II, in this order. These three types alone accounted for 67 per cent of the cases of lobar pneumonia. Types V, VII, VIII and IV ranked next in order of frequency among the cases of lobar pneumonia and together accounted for another 24 per cent. Eleven other types were distributed among the remaining 9 per cent of the cases. Types I and III were also most frequent among the cases of mixed pneumonia, and types V, VII and VIII followed in this order. The cases of atypical pneumonia showed a wider distribution of the various types of pneumococci. Type III was the most frequent and was followed in order by types VIII, V, I, X, VII, VI, XX, XVIII and II. These ten types together were responsible for 71 per cent of the cases of atypical pneumonia and the remaining cases were distributed among sixteen other types.

Within the groups of cases of pneumonia referred to the respective types, the relative proportions of cases of the different pulmonary lesions varied markedly. Thus, less than a fifth of the lesions associated with type I or II were atypical, whereas somewhat over a third of those associated with types III, IV, V and VII and considerably more than half of those due to the remaining types were atypical. Both the distribution of types according to the different pulmonary lesions and the relative proportions of cases of the same type showing the different kinds of pulmonary involvement were similar, in general, to the frequencies noted clinically.[2] The discrepancies are accounted for mainly by the differences in fatality rates among the different types and with the different pulmonary lesions. In particular, the higher death rate among the cases of atypical pneumonia due to the more common types results in a considerably greater proportion of such cases within the group of each type at autopsy than is observed clinically. It is pos-

sible, however, that this discrepancy is dependent in some measure on the difficulties of differentiating the various kinds of pulmonary lesions clinically in some cases, even with the aid of roentgenograms.

Sex and Age Distribution (Table 1).—The usual predominance of males over females was noted among the present cases. This predominance was greater among patients with lobar pneumonia (2.5 to 1) than among those with atypical pneumonia (1.7 to 1). Notable exceptions were the patients with atypical pneumonia due to types III and VIII, among which females predominated.[4]

Atypical pneumonia was considerably more frequent than lobar pneumonia among the very young and the very old. The pneumonic lesions due to type III in this series were almost entirely in persons over 50 years old. Furthermore, type III was most common among the patients in that age group regardless of the character of the pulmonary lesion. More than a third of the patients with lobar pneumonia due to types I, II, V or VII were between 20 and 49 years of age, whereas among the patients with atypical pneumonia a smaller proportion of those whose lesions were due to types I and II and a considerably greater proportion of those whose lesions were due to types V and VII were between 20 and 49 years old.

Duration of the Disease (Table 1).—The duration of illness from the time of onset of the pneumonia to the time of death was determined as nearly as possible from the clinical data. Among the persons with lobar pneumonia in whom this duration could be estimated, the majority died between the fifth and the ninth day, the largest number on the seventh day. The illness was of four days' duration or less in 11 per cent of the patients and of more than fourteen days' duration in 13 per cent. The serum-treated patients resembled those with lobar pneumonia in this respect. Among the persons with atypical pneumonia 21 per cent had an illness of four days or less, and 33 per cent were ill more than fourteen days. The prolonged illnesses were usually associated with focal purulent complications. More than half of the persons with mixed (lobar and atypical) pneumonia died on the sixth or the seventh day of the disease. The duration of the illness could not be determined in 43 per cent of the patients with atypical pneumonia as compared with 11 per cent of those with lobar pneumonia. This discrepancy can be accounted for in part by the more frequent occurrence of atypical pneumonia in the course of other serious illnesses, as will be noted subsequently, and by the fact that an insidious onset is more frequent with this type of lesion than with lobar pneumonia.

Extent of the Pulmonary Lesion (Table 1).—The pneumonia was bilateral in approximately one third of the patients with lobar pneumonia

4. Finland, M., and Sutliff, W. D.: Arch. Int. Med. **53**:481, 1934.

TABLE 1.—*Analysis of Data Obtained Clinically and at Autopsy in Six Hundred and Eighty-Four Cases of Pneumonia Associated with Specific Types of Pneumococci (Cooper)*

Pulmonary lesion.....	Lobar Pneumonia										Atypical Pneumonia														Mixed (Lobar and Atypical)				
Pneumococcus type...	I	II	III	IV	V	VII	VIII	Others	All Types	Serum-Treated	I	II	III	IV	V	VI	2VII	VIII	X	XVIII	XX	Others	All Types	I	III	Others	All Types	All Cases
Cases.....	104	43	81	14	32	17	17	31	339	44	21	11	47	9	22	15	16	28	19	12	14	76	290	13	11	31	55	684
Sex																												
Males.....	87	36	50	5	21	13	9	22	243	38	19	8	21	7	12	10	15	12	10	8	10	53	185	10	5	20	35	463
Females.....	17	7	31	9	11	4	8	9	96	6	2	3	26	2	10	5	1	16	9	4	4	23	105	3	6	11	20	221
Age, years																												
<1.....	0	0	0	1	0	0	1	0	2	0	4	0	0	0	3	2	0	0	0	1	1	6	17	0	0	0	0	19
1-11.....	2	0	0	0	0	0	0	0	2	0	1	1	1	0	1	0	0	0	1	0	1	6	12	0	0	1	1	15
12-19.....	0	1	0	0	1	0	1	1	4	3	0	1	2	1	0	0	0	6	0	0	1	2	7	1	0	0	1	12
20-29.....	6	4	0	2	1	0	1	2	16	3	1	0	0	1	2	4	6	0	4	1	0	1	20	1	0	4	5	41
30-39.....	16	4	4	1	4	1	0	6	36	12	0	1	2	0	1	2	1	2	1	0	0	6	16	1	2	4	7	59
40-49.....	25	9	5	3	7	5	3	5	62	10	1	0	4	2	5	0	1	3	3	2	4	10	35	4	0	3	7	104
50-59.....	15	13	21	5	9	6	6	9	84	7	2	4	11	3	1	2	2	8	2	2	1	20	58	1	1	5	7	149
60-69.....	24	9	30	2	8	2	2	5	82	8	6	0	11	0	6	1	0	5	2	5	3	12	51	1	6	8	15	148
70-79.....	15	2	16	0	2	2	1	3	41	1	4	3	11	0	2	4	6	7	4	1	3	10	55	3	2	5	10	106
80+.....	1	1	5	0	0	1	2	0	10	0	2	1	5	2	1	0	0	3	2	0	0	3	19	1	0	1	2	31
Lobes																												
Right																												
Lower.....	3	3	7	4	0	1	0	5	23	2	0	1	1	1	0	2	2	2	1	1	1	10	22	0	0	0	0	45
Middle.....	0	0	0	0	1	0	0	0	1	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	2
Upper.....	6	4	6	1	4	1	2	2	26	2	0	2	0	1	1	0	1	1	1	0	0	2	9	0	0	0	0	35
Lower and middle	1	0	3	1	3	1	0	3	12	1	0	0	1	0	0	0	0	1	0	0	0	1	3	0	0	0	0	15
Upper and middle	6	3	5	2	2	1	0	1	20	1	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	21
Upper and lower..	7	3	6	0	0	2	0	3	21	2	1	0	2	0	1	1	0	1	0	0	0	1	7	0	0	2	2	30
All 3 lobes.....	17	8	11	1	6	4	2	2	51	4	0	0	5	0	2	0	0	0	0	0	0	2	9	0	1	0	1	61
Left																												
Lower.....	9	3	6	1	2	3	2	2	28	3	2	0	1	0	2	0	0	4	2	0	0	3	14	0	0	0	0	42
Upper.....	6	0	2	1	2	0	0	2	13	4	1	0	1	0	1	0	0	0	0	0	0	4	7	0	0	0	0	20
Lower and upper.	10	5	13	0	3	1	1	2	35	3	1	0	1	0	0	1	0	0	1	0	0	3	7	1	0	0	1	43
Bilateral																												
2 lobes.....	11	4	9	1	3	1	6	6	41	4	8	3	9	4	4	1	6	11	5	5	7	13	75	3	2	5	10	126
3 lobes.....	13	4	10	1	4	1	3	3	39	4	1	1	4	1	3	1	0	4	1	2	0	5	23	1	1	7	9	71
4 lobes.....	9	4	2	1	0	1	1	0	18	6	2	0	0	0	3	2	0	0	0	0	1	1	9	3	4	7	14	41
5 lobes.....	6	2	1	0	2	0	0	0	11	8	4	3	16	2	4	5	2	5	5	3	2	27	78	5	3	10	18	107
Microscopic only....	..	..	..	..	..	..	..	..	..	..	1	1	6	0	1	2	4	0	2	1	3	4	25	..	..	..	..	25
Duration, days																												
1-3.....	4	1	5	1	1	0	2	0	14	1	3	3	4	0	4	0	0	2	2	1	0	2	21	0	0	0	0	35
4.....	6	1	4	0	5	1	1	1	19	4	0	1	2	0	2	1	1	2	1	2	1	1	14	1	1	2	4	37
5.....	9	6	4	2	2	2	2	3	30	9	0	0	2	0	2	2	1	1	2	0	0	5	15	0	1	0	1	46
6.....	8	6	4	2	1	1	2	2	26	5	2	0	2	0	1	1	1	3	0	0	1	3	14	4	2	7	13	53
7.....	16	7	11	2	4	4	1	3	48	7	1	0	2	0	1	0	1	1	0	0	0	2	8	2	4	6	12	68
8.....	11	1	16	1	3	2	1	3	38	2	0	0	1	0	2	0	0	2	2	0	0	4	11	3	1	2	6	55
9.....	8	4	5	0	3	3	1	2	26	3	0	0	2	0	0	0	3	0	0	0	1	0	0	6	0	1	3	4	36
10.....	7	5	6	1	2	0	0	1	22	5	0	0	0	0	1	0	0	0	1	0	1	2	5	1	0	0	1	28
11-14.....	15	4	8	2	3	2	1	4	39	3	2	2	3	0	1	0	2	1	1	0	1	5	18	0	0	2	2	59
15-21.....	9	3	5	1	2	1	1	2	24	5	2	1	4	0	4	0	1	3	2	2	0	8	27	1	0	1	2	53
22+.....	2	3	0	0	3	0	5	3	16	0	0	0	6	2	1	0	3	2	2	2	3	6	27	0	0	1	1	44
Not known.....	9	2	13	2	3	1	0	7	37	0	11	4	19	7	3	8	6	11	6	4	7	38	124	1	1	7	9	170

and in two thirds of those with atypical pneumonia. Among those with unilateral involvement, the right side was affected twice as frequently as the left in both those with lobar and those with atypical pneumonia, but multiple lobe involvement was relatively more frequent among those with unilateral lobar pneumonia than among those with unilateral atypical pneumonia. In almost all the patients with mixed (lobar and atypical) pneumonia the involvement was extensive and bilateral, and the lesion was usually different in each lung. In 25, or 9 per cent, of the patients with atypical pneumonia, the lesion was not detected in the gross examination of the lungs but was noted first during the study of the microscopic sections. When due allowance is made for the small numbers of cases, the locations of the pulmonary lesions showed no striking differences among the various types. At least among the common types, no special predilection for any given part of the lung could be made out. In the patients with lobar pneumonia, the pulmonary lesions were more extensive, as judged by the average number of lobes involved in those whose pneumonia was due to types I and II (average, 2.4 lobes), with less extension in those whose pneumonia was due to type IV or the less frequent types (average, 1.7 and 1.8 lobes, respectively) than in those whose lesions were due to the other common types (average, 2.1 or 2.2 lobes). It is difficult to estimate the extent of the lesions in the patients with atypical pneumonia since no attempt was made to quantitate the amount of consolidation within each lobe. The average number of lobes showing gross lesions, however, was greater in the patients with atypical than in those with lobar pneumonia and was greatest among those with mixed pneumonia (averages, 2.9, 2.2 and 3.7 lobes, respectively). Among the serum-treated patients, the average number of lobes involved was 2.8.

Complications (Table 2).—Most of the usual focal purulent pneumococcic complications were more common in the patients with lobar pneumonia than in those with atypical pneumonia. Peritonitis and involvements of the middle ear and mastoid were exceptions. Empyema, unilateral or bilateral, was present in 24 per cent of the patients with lobar pneumonia and in 11 per cent of those with atypical pneumonia. The highest incidence of this complication was in patients with lobar pneumonia from type I and in patients with pneumonia from type V with either kind of pulmonary lesion. Pericarditis, fibrinous or purulent, was found in 11 per cent of the patients with lobar pneumonia and in 6 per cent of those with atypical pneumonia. Vegetative endocarditis occurred in 6 per cent of the patients with lobar pneumonia and in 3 per cent of those with atypical pneumonia and was most frequent among those with lobar pneumonia due to types II and V. Meningitis was found in from 3 to 4 per cent of the patients irrespective of the character of their pulmonary lesions. It was most frequent in those

TABLE 2.—*Complications Among Six Hundred and Forty-Eight Cases of Typed Pneumococcic Pneumonia Studied During Life and at Autopsy*

Pulmonary lesion.....	Lobar Pneumonia										Atypical Pneumonia													Mixed (Lobar and Atypical)				
Pneumococcus type...	I	II	III	IV	V	VII	VIII	Others	All Types	Serum-Treated	I	II	III	IV	V	VI	2VII	VIII	X	XVIII	XX	Others	All Types	I	III	Others	All Types	All Cases
Cases.....	104	43	81	14	32	17	17	31	339	44	21	11	47	9	22	15	16	28	19	12	14	76	290	13	11	31	55	684
Empyema																												
Unilateral.....	24	8	15	3	7	4	3	5	69	14	4	2	6	0	3	1	1	4	1	1	1	4	28	3	1	5	9	106
Bilateral.....	4	1	1	0	4	1	1	2	14	0	0	0	0	0	3	1	0	0	0	0	0	0	4	0	0	0	0	18
Pericarditis.....	12	4	5	0	6	3	2	3	35	2	1	0	2	2	0	4	0	1	2	1	0	5	18	2	1	0	3	56
Endocarditis.....	3	6	2	0	4	2	1	2	20	3	1	0	2	0	0	2	0	0	1	2	0	2	10	0	0	1	1	31
Meningitis.....	1	5	0	0	1	2	1	2	12	1	0	0	3	1	0	0	1	1	1	0	1	2	10	0	1	1	2	24
Lung abscesses																												
Gross.....	8	4	13	2	1	1	2	3	34	4	1	1	7	0	4	2	0	2	3	1	3	7	31	0	4	3	7	72
Microscopic.....	6	4	13	1	3	0	0	0	27	4	1	0	2	0	0	2	0	1	0	1	0	2	9	1	2	2	5	41
Peritonitis.....	1	0	0	0	1	0	1	1	4	0	0	1	2	0	2	0	0	1	0	1	1	0	8	0	0	0	0	12
Otitis and/or mastoiditis.....	0	1	2	1	0	0	0	0	4	0	0	0	1	2	2	0	1	0	0	0	1	1	8	0	0	0	0	12
Bronchitis.....	19	7	14	4	6	4	3	9	66	8	4	1	10	2	7	2	4	5	2	1	1	8	47	3	5	6	14	127
Tracheobronchitis....	39	10	37	4	13	6	6	10	125	15	10	4	19	5	5	6	6	11	10	5	3	27	111	8	5	12	25	261
Focal infection with Str. haemolyticus..	0	0	0	0	0	1	1	1	3	2	0	0	2	0	1	0	1	0	2	0	0	4	10	0	0	1	1	14
Urinary infection.....	6	1	2	0	1	0	1	0	11	1	3	0	5	1	1	0	0	0	1	0	0	2	13	1	0	3	4	28
Decubitus ulcers.....	1	2	1	0	0	0	1	0	5	2	0	2	3	2	3	1	0	1	0	2	2	1	17	1	0	1	2	24
Pleural effusion (sterile)																												
Unilateral.....	13	8	15	3	6	2	0	4	51	3	2	1	4	2	1	1	2	0	3	2	1	6	25	0	0	4	4	80
Bilateral.....	4	3	0	1	0	0	0	1	9	2	0	0	3	0	3	1	2	0	0	1	0	4	14	0	1	2	3	26
Pulmonary atelectasis.....	8	4	2	1	4	0	1	2	22	4	3	0	3	1	4	3	1	0	0	2	0	7	24	1	0	1	2	48
Pulmonary thrombosis and infarcts....	3	2	3	0	0	0	2	1	11	1	2	1	5	0	3	2	0	2	2	2	0	2	21	1	0	3	4	36
Resolution.....	4	2	4	1	2	1	3	0	17	2	0	0	1	0	0	0	0	0	0	0	0	1	2	0	0	1	1	20
Organization.....	5	6	7	4	2	1	3	6	34	2	1	1	6	1	0	4	0	2	2	3	5	12	37	0	0	4	4	75
Fibrinous pleuritis....	75	37	60	10	28	12	13	22	257	37	9	4	18	5	13	1	6	11	6	4	4	23	104	10	8	24	42	403
Cardiac dilatation																												
Right.....	13	6	6	2	5	2	1	0	35	6	0	3	3	1	0	3	2	2	1	3	2	5	25	0	0	1	1	61
Left.....	0	1	0	0	1	1	0	1	4	3	0	1	2	2	0	0	0	1	0	0	1	0	1	8	0	0	2	2	14
Right and left.....	9	2	2	3	1	0	3	1	21	2	2	0	5	0	2	1	0	1	1	0	0	2	14	2	2	4	8	43
Jaundice.....	1	3	5	1	5	1	1	1	18	1	0	1	2	0	1	1	0	0	2	0	1	2	10	0	0	1	1	29
Central necrosis of liver.....	13	6	4	2	0	1	1	1	28	1	2	1	3	0	1	3	0	1	1	0	4	10	26	0	2	1	3	57
Acute nephritis.....	2	1	0	0	0	0	1	0	4	0	0	0	1	0	0	0	1	0	1	1	0	2	6	0	0	1	1	11
Infarcts and thromboses (nonpulmonary).....	1	1	1	1	2	0	0	2	8	2	3	1	0	1	2	1	0	1	2	2	0	1	14	1	0	3	4	26

Less Frequent Complications, with Types and Numbers of Cases

Lobar pneumonia: suppurative parotitis, I; subcutaneous abscesses, II; mediastinitis, I; acute pancreatitis, III; abscesses of the liver and kidney, III; agranulocytosis, IV; purpura, IV—1 case each.

Atypical pneumonia: subcutaneous abscess, XVIII; focal encephalitis, VII; subphrenic abscess, XX—1 case each.

Mixed (lobar and atypical): mediastinitis, I—1 case.

with lobar pneumonia from type II and in those with atypical pneumonia from type III. Peritonitis occurred less often. It was more frequent in patients with atypical pneumonia and in patients with type V infections. Suppuration of the middle ear and mastoid was also more frequent in the patients with atypical pneumonia. The incidence of the latter complication and of meningitis is probably higher than indicated because permission to examine the head was not obtained in a large proportion of the cases. Pulmonary abscesses were noted in 18 per cent of the patients with lobar pneumonia and in 14 per cent of those with atypical pneumonia. This term is used here to designate necrosis of the parenchyma of the lung and coalescence of adjacent alveoli and their exudate without peripheral fibrous tissue reaction. A considerable proportion of these abscesses were noted only in the microscopic sections of the affected lungs. The highest incidence of this complication was in patients with pneumonia from type III,[4] 30 per cent of whom showed such lesions. Bronchitis and tracheobronchitis were noted in more than half the patients and were equally common with all types of pneumococci and with the different kinds of pneumonia. In most of the cases the nonconsolidated portions as well as the pneumonic part of the lung showed inflammation of the bronchi. The incidence of the complications mentioned and of the others noted in the table in the patients with mixed pneumonia was usually intermediate between that in the patients with lobar and that in the patients with atypical pneumonia.

Acute nonpurulent or nonpneumococcic complications are also listed in table 2. Among the latter are included focal infections with beta hemolytic streptococci and urinary infections, mostly with colon bacilli. Mention needs to be made of only a few of the more common complications which are of particular interest in relation to the different pulmonary lesions or types of pneumococci. Fibrinous pleurisy was present in three fourths of the patients with lobar pneumonia and in only about a third of those with atypical pneumonia. The incidence of this lesion was remarkably similar for each of the types of pneumococci with the exception of type V in association with atypical pneumonia; among patients with atypical pneumonia of the latter type the lesion was almost as frequent as among those with lobar pneumonia. Atelectasis, partial or complete, of varying portions of the lung was equally frequent in patients with lobar and in those with atypical pneumonia. In some cases this occurred in lobes undergoing resolution, and in others it was noted in lobes which gave no evidence of previous involvement.[5] Pulmonary infarcts and thromboses of pulmonary vessels were more common in patients with atypical pneumonia. The highest incidence was in those with pneumonia due to type III.

5. Finland, M., and Loverud, H. I. L.: Ann. Int. Med., to be published.

Associated Diseases and Conditions (Table 3).—Only the more common conditions are listed in the body of table 3. The less frequent ones are mentioned in the footnotes. The significant conditions fall into three main groups: The first group includes chronic diseases of the lung—emphysema, tuberculosis and chronic nontuberculous pulmonary infections. These were more frequently associated with atypical than with lobar pneumonia, and with type III more frequently than with other types. Types VIII, X and XVIII were frequent with atypical pneumonia in this group. Fibrous pleurisy may also be noted here. This condition was usually old and extensive and about as frequently bilateral as unilateral. It was noted in about half of the patients with atypical pneumonia and in almost two thirds of those with lobar pneumonia. This finding is noteworthy in view of the recent interest in the pneumothorax treatment of pneumonia.

The second group of associated conditions includes mainly a number of chronic nonpulmonary diseases, the more common of which are: (1) chronic cardiovascular disease of all kinds, (2) congestive heart failure from all causes but mostly from degenerative heart disease, (3) chronic Bright's disease, (4) chronic urinary infections, mostly pyelonephritis and pyelitis, (5) various types of cirrhosis of the liver and (6) chronic gastro-intestinal ulcers. Lobar pneumonia and atypical pneumonia occurred with similar frequency in this group, except among the patients with hypertensive heart disease, in whom atypical pneumonia was more common. There was no special predominance of types in this group, except that type III was most frequent among patients with congestive heart failure.

The third group comprises the more acute and serious illnesses, in most of which the pneumonia may be considered secondary. The following diseases are included: (1) coronary occlusions or acute cardiac infarctions, (2) cerebrovascular accidents (hemorrhage, embolus and thrombosis), (3) malignant diseases, mostly terminal, with extensive metastases, (4) a variety of acute infections, local and general, and serious injuries (most of these are given in the footnotes in table 3), (5) pregnancy (usually accompanied by miscarriage) or the puerperium and (6) postoperative pneumonia. In this group atypical pneumonia was considerably more frequent than lobar pneumonia, and the more common types of pneumococci did not predominate. Notable exceptions within this group were the cases in which pneumonia complicated pregnancy and the puerperium, in which the pneumonia was predominantly lobar and due to the more common types of pneumococci.

Bacteriologic Findings (Table 4).—Cultures were made of various materials obtained during life in about two thirds of the cases of lobar pneumonia but in only about a third of those of atypical pneumonia. The homologous specific pneumococcus was identified in one or more

TABLE 3.—*Significant Associated Diseases and Conditions Among Six Hundred and Eighty-Four Cases of Typed Pneumococcic Pneumonia Studied During Life and at Autopsy*

Pulmonary lesion.....	Lobar Pneumonia										Atypical Pneumonia														Mixed (Lobar and Atypical)				
Pneumococcus type...	I	II	III	IV	V	VII	VIII	Others	All Types	Serum-Treated	I	II	III	IV	V	VI	2VII	VIII	X	XVIII	XX	Others	All Types	I	III	Others	All Types	All Cases
Cases.....	104	43	81	14	32	17	17	31	339	44	21	11	47	9	22	15	16	28	19	12	14	76	290	13	11	31	55	684
Chronic bronchitis and/or bronchiectasis.....	3	2	7	1	1	0	0	0	14	1	2	2	7	1	1	0	2	1	1	3	2	6	28	1	0	2	3	45
Emphysema.....	10	0	7	2	1	1	0	3	24	1	2	0	6	1	2	1	0	4	6	3	1	7	33	2	0	2	4	61
Pulmonary tuberculosis.....	4	0	1	0	2	1	0	0	8	1	0	0	4	0	0	2	2	3	0	2	2	8	23	1	0	1	2	33
Fibrous pleurisy																												
Unilateral.....	37	19	29	6	8	3	1	11	114	16	5	1	16	3	7	4	5	3	5	1	3	20	73	2	2	6	10	197
Bilateral.....	35	11	22	6	8	6	0	10	98	14	5	4	16	3	7	7	6	0	8	5	6	18	85	4	5	13	22	205
Cardiovascular disease																												
Rheumatic.....	3	4	0	0	2	0	2	5	16	2	1	2	2	0	0	1	0	2	2	1	0	3	14	1	0	0	1	31
Syphilitic.....	3	1	2	0	0	0	2	1	9	3	0	0	1	0	0	1	1	0	1	1	0	1	6	0	0	2	2	17
Hypertensive.....	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	1	2	1	0	0	2	7	0	0	1	1	9
Coronary occlusion...	1	2	1	0	1	1	0	0	6	1	2	0	3	0	0	0	0	3	2	1	0	1	12	0	2	2	4	22
Congestive failure....	1	1	10	2	3	1	1	1	20	0	2	2	5	0	0	0	0	7	2	0	0	4	22	0	1	2	3	45
Cerebrovascular accident.....	1	0	1	0	0	2	1	1	6	0	2	1	2	1	1	0	0	4	2	1	1	4	19	1	1	1	3	28
Chronic nephritis.....	3	3	4	1	1	0	1	3	16	0	1	0	1	1	3	0	2	3	1	0	0	4	16	0	0	0	0	32
Chronic urinary infection.....	7	3	5	0	3	1	0	1	20	2	2	1	4	0	0	1	1	0	2	2	0	8	21	0	1	1	2	43
Cirrhosis of liver.....	9	7	3	2	0	0	0	8	29	4	3	1	2	1	1	1	2	0	3	1	1	4	20	1	2	4	7	56
Malignancy																												
Primary in lung..	1	1	2	0	0	0	0	0	4	0	1	0	0	0	1	1	0	0	0	0	0	3	6	0	0	0	0	10
All others.....	2	0	1	0	1	3	2	1	10	0	2	1	6	0	1	2	4	2	5	3	3	8	37	1	1	2	4	51
Gastro-intestinal ulcers.....	1	2	3	0	1	2	0	0	9	1	0	0	2	0	0	0	0	1	0	1	1	2	7	0	0	2	2	18
Pregnancy and/or parturition.....	2	0	1	2	3	0	5	0	13	2	0	0	0	0	1	0	0	2	0	0	0	3	6	0	0	2	2	21
Recent surgical operation.....	0	0	0	0	0	1	1	1	3	0	1	0	2	0	1	0	0	2	4	0	0	10	20	0	0	0	0	23

Other Associated Conditions, with Types and Number of Cases

Lobar pneumonia: adhesive pericarditis, I, 2 cases; VIII, VI and IX, 1 case each; nephrolithiasis, II, 2 cases; X, 1 case; aniline poisoning, I, 1 case; intestinal obstruction, V, 1 case; exfoliative dermatitis, VII, 1 case; subacute bacterial endocarditis, XIX, 1 case.

Atypical pneumonia: general peritonitis, IV, 1 case; XX, 1 case; acute hemorrhagic encephalitis, VI, 1 case; meningococcic meningitis, VI, 1 case; acute pancreatitis, XI, 1 case; dementia paralytica, I, 1 case; acute bulbar poliomyelitis, III, 1 case; XIV, 1 case; pemphigus, VIII, 1 case; XII, 1 case; tuberculous meningitis, VII, 1 case; thrush, VI, 1 case; influenzal meningitis and fracture of the skull, XI, 1 case; XXIII, 1 case; sepsis due to Staph. aureus, XIV, 1 case; pelvic abscess, XX, 1 case; pertussis, XX, 1 case; congenital heart disease, XI, 1 case; perforated esophagus and mediastinitis, VI, 1 case; gangrene of the leg, X, 1 case; XIV, 1 case; periarteritis nodosa, XIV, 1 case; pernicious anemia, III, 1 case; negative for types I-XX, 1 case; agranulocytosis, III, 1 case; XII, 1 case; Addison's disease, X, 1 case; hemoglobinuria, IX, 1 case; burns, XXVIII, 1 case; adhesive pericarditis, VIII, 1 case; XI, 1 case; XII, 1 case; hemolytic jaundice, VII, 1 case; intestinal obstruction, VII, 1 case; nephrolithiasis, VII, 2 cases; lipoid nephrosis, XX, 1 case; lipoid pneumonia, XX, 1 case.

Mixed (lobar and atypical): diabetic nephrosis, V, 1 case.

Pulmonary lesion	Lobar Pneumonia										Atypical Pneumonia													Atypical				
Pneumococcus type	I	II	III	IV	V	VII	VIII	Others	All Types	Serum-Treated	I	II	III	IV	V	VI	2VII	VIII	X	XVIII	XX	Others	All Types	I	III	Others	All Types	All Cases
Cases	104	43	81	14	32	17	17	31	339	44	21	11	47	9	22	15	16	28	19	12	14	76	290	13	11	31	55	684
Occurrence of Pneumococci of the Homologous Type in Cultures of Various Materials																												
1. Material obtained during life																												
Sputum																												
Cultures made	68	26	41	5	22	9	8	21	200	44	2	0	17	1	7	3	6	7	5	2	3	29	82	7	4	15	26	308
Cultures showing homologous type	60	24	39	5	20	7	7	17	179	42	2	..	13	1	5	3	6	6	5	2	3	25	71	7	4	15	26	276
Blood																												
Cultures made	65	29	42	9	20	12	9	21	207	43	2	2	14	2	6	4	4	8	3	1	4	22	72	8	4	18	30	309
Cultures showing homologous type	52	24	27	6	15	9	6	5	144	32	2	1	4	1	2	0	1	3	0	1	2	3	20	4	3	12	19	183
Exudates																												
Cultures made	6	7	3	0	5	4	1	3	29	3	1	2	5	1	4	2	3	1	1	1	1	11	33	1	1	1	3	65
Cultures showing homologous type	4	5	3	..	4	4	1	2	23	2	1	2	3	0	3	0	2	1	0	1	0	2	15	1	0	1	2	40
2. Material obtained at autopsy																												
Cardiac blood																												
Cultures made	93	38	72	13	29	15	16	29	305	39	19	10	41	7	17	14	15	25	16	11	13	68	256	13	9	30	52	613
Cultures showing homologous type	49	23	55	8	21	8	7	13	184	16	11	3	20	3	10	3	3	7	3	3	1	21	88	6	5	20	31	303
Lungs																												
Cultures made	91	34	74	13	28	16	13	29	298	39	14	10	32	9	16	9	10	22	18	7	13	58	218	11	11	28	50	566
Cultures showing homologous type	79	27	70	9	26	15	12	25	263	31	14	9	29	9	15	8	9	20	11	6	12	50	192	10	11	25	46	501
Exudates																												
Cultures made	32	9	24	3	11	7	7	9	102	11	4	5	16	5	8	8	5	8	8	6	5	21	99	3	1	9	13	214
Cultures showing homologous type	23	8	21	2	11	6	5	6	82	9	4	4	13	2	7	3	4	4	3	3	2	7	56	3	1	5	9	147
Occurrence of Other Significant Organisms and Coexistence of Pneumococci of the Homologous Type in the Same Materials																												
Cases with other organisms	39	11	21	8	11	7	6	14	117	24	6	7	22	5	5	9	9	12	15	5	8	40	143	6	4	13	23	283
1. Material obtained during life																												
Sputum																												
Cultures of other organisms	8	1	4	1	0	3	0	6	23	6	0	0	2	0	3	0	1	1	1	1	2	9	20	1	1	3	5	48
With homologous type	3	0	4	1	..	1	..	4	13	5	..	..	0	..	1	..	1	1	1	1	2	5	12	1	1	2	4	29
Blood																												
Cultures of other organisms	3	1	0	0	0	1	0	3	8	4	0	0	1	0	0	0	1	0	0	0	0	4	6	1	0	0	1	15
With homologous type	2	1	..	..	..	0	..	0	3	3	..	..	0	..	..	..	0	..	..	..	..	0	0	1	..	..	1	4
Exudates																												
Cultures of other organisms	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	2	2	0	0	0	0	2
With homologous type	..	..	..	..	..	..	..	..	..	0	..	..	..	..	..	..	..	..	..	..	..	0	0	..	..	..	..	0
2. Material obtained at autopsy																												
Cardiac blood																												
Cultures of other organisms	2	1	4	4	1	1	1	1	15	3	1	1	3	0	1	3	3	2	4	0	1	12	31	0	0	4	4	50
With homologous type	1	0	2	3	0	0	0	0	6	0	0	0	0	..	0	1	0	0	0	..	0	1	2	..	..	1	1	9
Lungs																												
Cultures of other organisms	31	10	12	8	10	5	4	7	87	23	6	7	19	5	3	6	7	10	14	4	7	32	120	6	4	13	23	230
With homologous type	22	9	9	5	7	4	4	3	63	20	5	6	12	5	1	3	6	8	8	3	6	23	86	5	4	11	20	169
Exudates																												
Cultures of other organisms	9	1	6	2	1	2	2	3	26	3	0	2	6	1	1	4	2	3	5	1	2	12	39	1	0	4	5	70
With homologous type	2	0	2	1	1	0	1	1	8	0	..	2	4	0	0	0	1	1	0	0	0	2	10	0	..	0	0	18
Other Organisms Cultured (All Sources)																												
Pneumococci of other types*	6[a]	3[b]	2[c]	1[d]	1[e]	1[f]	0	2[g]	16	3	1[h]	0	1[i]	0	2[k]	0	0	0	1[l]	0	0	1[m]	6	1[n]	0	2[o]	3	25
Beta hemolytic streptococci	9	4	7	2	4	4	3	2	35	7	2	4	5	0	2	2	4	1	6	1	2	19	48	2	2	4	8	91
Streptococcus viridans	8	3	3	1	5	1	2	7	30	12	0	2	3	2	1	2	2	3	1	2	1	8	27	2	2	1	5	62
Staphylococcus aureus-haemolyticus	10	3	8	3	5	5	3	5	42	13	0	3	9	2	1	4	6	5	11	3	1	17	62	1	1	4	6	110
Bacillus mucosus-capsulatus	4	0	1	1	0	0	0	1	7	3	0	0	0	0	0	0	0	1	0	0	0	1	2	0	0	2	2	11
Tubercle bacilli (stained smears)	2	0	0	0	0	1	0	0	3	1	0	0	4	0	0	2	2	2	0	2	2	3	17	1	0	0	1	21
Haemophilus influenzae	3	1	4	2	3	1	0	3	17	2	1	2	2	2	1	0	3	3	2	1	1	9	27	0	0	5	5	49
Miscellaneous organisms†	17	5	9	4	3	4	1	0	43	11	2	4	9	5	2	7	2	5	8	3	5	16	68	3	0	7	10	121

* Types (cf. superscripts): (a) VI, X, XIV, XXI, XXIV, XXX; (b) III, VIII, XIX; (c) VIII, XXIV; (d) XIII; (e) X; (f) IX; (g) III, a pneumococcus showing no agglutination with serums for I to XXXII; (h) XX; (j) XII; (k) XIV, XVIII; (l) XIX; (m) XXIX; (n) III; (o) XX, a pneumococcus showing no agglutination with serums for I to XXXII.

† Most of these were Escherichia coli, Bacillus Welchii and Micrococcus catarrhalis, but also included were: meningococcus and Bacillus typhosus, each in 1 case of type V lobar pneumonia, and Bacillus paratyphosus B in 1 case of type V and 1 case of type VI mixed pneumonia.

of the samples of sputum in almost every case in which material was obtained, i. e., in 90 per cent of the cases of lobar pneumonia, in 87 per cent of those of atypical pneumonia and in all of those of mixed pneumonia. One or more blood cultures made during life yielded the homologous pneumococcus in 70 per cent of the cases of lobar pneumonia, but in only 28 per cent of the cases of atypical pneumonia. Of the cases of mixed (lobar and atypical) pneumonia in which blood was cultured during life, 45 per cent yielded the homologous pneumococcus. Purulent exudates, mostly pleural and spinal fluids, were obtained for culture during life in only a small number of cases. The homologous pneumococcus was identified in such exudates in 80 per cent of the cases of lobar pneumonia and in 45 per cent of cases of atypical pneumonia in which exudates were examined.

Cultures were made at autopsy in most of the cases. These yielded the respective homologous types of pneumococci in a proportion of cases similar to that noted in regard to antemortem cultures. Thus, 60 per cent of the cases of lobar pneumonia yielded cultures of the respective homologous types of pneumococci from cardiac blood as compared with 34 per cent of the cases of atypical pneumonia and 58 per cent of the cases of mixed pneumonia. Cultures from the consolidated lobes were positive for the respective homologous types of pneumococci in 81 per cent of cases of lobar, 88 per cent of cases of atypical, and 92 per cent of cases of mixed pneumonia. These cultures from the lungs were frequently the only source of the pneumococcus, particularly in cases of atypical pneumonia. Purulent exudates obtained at autopsy yielded positive cultures for the respective homologous types of pneumococci in 80 per cent of the cases of lobar, 56 per cent of the cases of atypical, and 70 per cent of the cases of mixed pneumonia, from which cultures of such materials were made. In the remaining cases, cultures from all these various sources were sterile or yielded other organisms. Such organisms were also frequently encountered in specimens which yielded the homologous types of pneumococci.

Other significant organisms, including heterologous types of pneumococci, were identified from one or more sources in a third of the cases of lobar pneumonia, in about half of the cases of atypical pneumonia and in 42 per cent of the cases of mixed pneumonia. Only the heterologous types of pneumococci and Friedländer's bacillus were more frequent among the cases of lobar pneumonia, while all the other organisms encountered were more frequent in the cases of atypical pneumonia. Staphylococci, streptococci and influenza bacilli were the most common among all the kinds of pneumonia. The majority of cases in which other organisms were cultured from sputum or from the consolidated lobes showed pneumococci of the respective homologous types in cul-

tures of the same material. When the other organisms were cultured from the blood or from exudates, cultures of the same materials yielded the homologous organisms less frequently. The frequency with which heterologous organisms were cultured was very similar among the cases of each of the common pneumococcus types.

CASES WITHOUT PNEUMONIA

In addition to the 684 cases in which various types of pneumococci were present with pneumonia, which have just been reviewed, there were 80 cases in which pneumococci were cultured from various sources and typed, but in which no evidence of pneumonia attributable to the types isolated was found at autopsy. In 62 of these cases the pneumococci were identified in cultures of infected exudates or of blood from the heart. Some of the relevant data in these cases are noted in table 5. In the remaining 18 cases, which are listed in table 6, the pneumococci were isolated in cultures from sputum, throat or lung, but they did not appear to be related to the pathologic changes.

Focal Infections and Sepsis (Table 5).—The most frequent types in focal infections and sepsis were types III and II, with 17 and 8 cases, respectively. Types I, V and VIII were each represented by 5 cases, and types XI, VII and IV, by 4, 3 and 2 cases, respectively. In each of the remaining 13 cases there was a different type. In 2 of the cases in which type III was isolated, other types were also obtained from the same source; type XXIV in one, and a pneumococcus which failed to agglutinate in serums for types I to XX in the other. In 2 other cases types of pneumococci, presumably unrelated to the infection, were identified in addition to the type obtained from the purulent focus: type III was cultured from sputum in a case of empyema due to type IV, and a pneumococcus that failed to agglutinate in serums for types I to XX was cultured from the lung in a case of peritonitis due to type VIII. The distribution of types among these cases, when due allowance is made for the relatively small numbers of cases, was similar to that among the cases in which atypical pneumonia was present.

Meningitis was the most common pneumococcic infection in this group; it was present in 18 cases, including 7 with type III, 2 each with types II and VII and the remaining 7 each with a different type. The meningitis in 7 instances was associated with infections of the middle ear and mastoid, in 2 cases with injuries of the head and infections of accessory sinuses, in 1 case with spina bifida and in 1 case with a meningococcic infection following intrathecal serum therapy. There was 1 case in which abscess of the brain (type III) was associated with infection of the sphenoid sinus. Pneumococcic infection of the middle ear and mastoid without meningitis was present in 5 cases (type V, 2

TABLE 5.—*Summary of Findings Among Sixty-Two Cases of Focal Infection or Bacteremia (Sepsis) with Different Types of Pneumococci, in Which Autopsy Showed No Evidence of Pneumonia*

Case	Sex	Age	Pneumococcic Infection	Type	Sources of Pneumococci	Other Organisms	Sources	Comment
1	F	68	Pulmonary abscess, endocarditis	I	Cardiac blood, abscess	0	.....	Probably postpneumonic
2	F	44	Pyohemothorax	I	Pleural fluid	0	.....	Cancer of lung with bronchial fistula
3	F	6 mos.	Sepsis	I	Cardiac blood	0	.....	No focus found (brain examined)
4	F	52	Sepsis	I	Cardiac blood	0	.....	Carcinoma of pancreas with metastases in liver
5	F	71	Sepsis	I	Cardiac blood	0	.....	Cardiac infarct, congestive failure, anemia
6	M	2 mos.	Sepsis	II	Cardiac blood	0	.....	Congenital syphilitic cirrhosis of liver, ascites
7	F	4	Otitis media, mastoiditis	II	Ear, mastoid	Staph. aureus	Ear, meninges	
8	F	2 days	Sepsis	II	Cardiac blood	0	.....	Jaundice; pneumococci seen in microscopic sections
9	M	56	Meningitis	II	Throat, blood, meninges	0	.....	
10	F	60	Sepsis	II	Cardiac blood	Esch. coli	Lung, kidney	Chronic pyelitis and cystitis, nephrolithiasis
11	F	16	Peritonitis	II	Blood, peritoneum, pleura	Str. haemolyticus, Staph. aureus, Esch. coli	Lung, cervix	Acute pancreatitis, old rheumatic condition of heart
12	F	22	Ethmoiditis, meningitis	II	Ethmoid, meninges	0	.....	Injury with abrasion of orbit
13	F	23	Septic abortion	II	Uterus	Miscellaneous	Uterus, peritoneum	General peritonitis
14	F	40	Cystitis	III, Neg. I-XX*	Urinary bladder	Esch. coli	Peritoneum	Perforated duodenal ulcer
15	M	31	Peritonitis	III	Peritoneum	Miscellaneous	Peritoneum	Perforated duodenal ulcer, subphrenic abscess
16	M	4	Otitis, mastoiditis, meningitis	III	Ear, spinal fluid, meninges	Str. haemolyticus	Lungs	
17	M	27	Otitis, mastoiditis, meningitis	III	Blood, ear, mastoid, spinal fluid, meninges	0	.....	
18	F	21	Pancreatic abscess	III	Cardiac blood, abscess	0	.....	
19	M	56	Peritonitis	III	Peritoneum	Str. haemolyticus, Staph. aureus, Esch. coli	Lung, peritoneum	Ruptured appendix with abscess formation
20	M	20	Mastoiditis	III	Mastoid	Str. haemolyticus	Blood, pleura, mastoid, meninges	Streptococcic meningitis following pneumococcic mastoiditis
21	M	42	Empyema of gallbladder	III	Gallbladder	0	.....	
22	M	66	Meningitis	III	Cardiac blood, lung, meninges	Staph. aureus	Lung	
23	F	7	Meningitis	III	Spinal fluid, meninges	0	.....	Serous otitic exudate
24	F	39	Peritonitis	III	Peritoneum	Esch. coli	Peritoneum	Pyelonephritis with perforation
25	F	70	Empyema of pleura	III	Cardiac blood, pleurae	0	.....	Adenocarcinoma of colon, endocarditis (marantic?)
26	M	70	Sepsis	III, XXIV	Cardiac blood	0	.....	Bronchogenic carcinoma with metastasis; jaundice, ascites
27	M	60	Brain abscess, sphenoiditis	III	Sphenoid	0	.....	
28	M	23	Otitis, meningitis	III	Ear, spinal fluid, meninges	0	.....	Focal encephalitis
29	M	25	Sphenoiditis, meningitis	III	Blood, spinal fluid	0	.....	Fractured skull
30	M	26	Sagittal sinus thrombosis, meningitis	III	Sinus, spinal fluid	Staph. aureus, Str. viridans	Lung	Bronchopneumonia (not pneumococcic)

31	M	35	Empyema of pleura	IV	Pleura	Str. haemolyticus and viridans, H. influenzae	Cardiac blood, pleura	Pneumococcus type III found in sputum; jaundice
32	M	40	Sepsis	IV	Cardiac blood	0	.....	Hemochromatosis
33	M	55	Bronchiectatic abscess	V	Abscess	Staph. aureus, Esch. coli	Abscess	
34	F	6	Otitis media	V	Right ear	Str. haemolyticus	Both ears, cardiac blood, meninges	
35	M	18 mos.	Otitis, mastoiditis	V	Mastoid	Str. haemolyticus	Blood, mastoid	
36	M	80	Empyema	V	Pleural fluid	Str. haemolyticus, Staph. aureus	Lung, pleural fluid	Bronchogenic carcinoma with local necrosis
37	F	28	Pelvic peritonitis	V	Blood, uterus, peritoneum	0	.....	Endometritis
38	M	20 mos.	Meningitis	VI	Meninges	Str. viridans, Esch. coli	Meninges	Extensive burns
39	F	65	Otitis, meningitis	VII	Cardiac blood, ear, meninges	0	.....	
40	M	77	Bronchiectatic abscess	VII	Abscess	0	.....	
41	M	60	Meningitis	VII	Cardiac blood, meninges	Meningococcus	Spinal fluid, meninges	
42	F	5	Peritonitis	VIII	Cardiac blood, peritoneum, liver, spleen	Pneumococcus Neg. I-XX*	Lungs	Pyelonephritis, nephrolithiasis, chronic infectious cirrhosis of liver, jaundice
43	F	25	Pyelonephritis, perinephritic abscess, endocarditis, peritonitis	VIII	Vegetation, pericardium, abscess, peritoneum	0	.....	Probably followed pyelonephritis
44	M	52	Sepsis	VIII	Cardiac blood	0	.....	Cirrhosis of liver
45	M	43	Subhepatic abscess	VIII	Abscess, peritoneum	Esch. coli	Abscess, peritoneum	Adenocarcinoma of colon with perforation
46	M	67	Peritonitis	VIII	Cardiac blood, peritoneum	0	.....	Gastrostomy for carcinoma of stomach
47	M	8 mos.	Empyema	IX	Pleural fluid	Staph. aureus	Cardiac blood, lung, pleura	Probably staphylococcic sepsis
48	M	49	Endocarditis	X	Vegetation	0	.....	Syphilitic aortitis, embolic nephritis
49	F	65	Hepatic abscess	XI	Abscess	H. influenzae, Esch. coli	Abscess	Chronic cholecystitis, portal thrombophlebitis
50	F	15 mos.	Otitis, meningitis	XI	Cardiac blood, ear, meninges	0	.....	
51	F	65	Inguinal adenitis	XI	Inguinal node	Esch. coli	Kidney	Also had pyelitis (not pneumococcic)
52	F	63	Sepsis	XI	Cardiac blood	0	.....	Diabetes, fatty liver
53	F	48	Meningitis	XII	Spinal fluid, cardiac blood, meninges	Staph. aureus	Lung	
54	M	71	Sepsis	XVIII	Cardiac blood	0	.....	Carcinoma of bile ducts, acute pancreatitis, jaundice
55	M	10 mos.	Otitis, meningitis	XIX	Ears, mastoids	0	.....	
56	M	2 mos.	Sepsis	XX	Cardiac blood	Dysentery bacilli	Kidney	Dysentery, pyelonephritis
57	M	50	Peritonitis	XXI	Peritoneum	Esch. coli	Peritoneum	Adenocarcinoma of stomach with perforation
58	M	50	Otitis, meningitis	XXII	Spinal fluid, cardiac blood, ear, meninges	0	.....	
59	M	4	Meningitis	XXV	Spinal fluid, cardiac blood, meninges	0	.....	
60	F	14 days	Meningitis	XXVIII	Cardiac blood, lungs, meninges	Miscellaneous	Cardiac blood, lung, meninges	Spina bifida, internal hydrocephalus
61	M	51	Otitis media	XXIX	Ear	0	.....	Carcinoma of stomach, extensive metastases in liver
62	M	64	Pyelonephritis, sepsis	Negative I-XX*	Cardiac blood, kidney, spleen	0	.....	Also had cirrhosis of liver

* The pneumococcus isolated failed to show agglutination with serums for types I to XX.

cases; types II, III and XXIX, 1 each). Death in 4 of these cases was associated with streptococcic or staphylococcic meningitis and sepsis, and pneumococci could not be recovered from the blood or the meninges. In the fifth case, an infection of the ear due to type XXIX was an incidental finding at autopsy in a patient who died of carcinoma of the stomach.

Peritonitis occurred in 9 cases and abscesses of abdominal organs in 4 additional cases. In these cases peritonitis was mainly secondary to perforations of viscera and to abdominal operations or was an extension from urinary or uterine infection. There was a case in which empyema of the gallbladder was associated with type III, a case in which hepatic abscess was associated with type XI, probably resulting from cholecystitis, a case in which pancreatic abscess was associated with type III and a case in which a postabortion uterine infection was associated with type II. Infections of the urinary tract occurred in 2 cases—cystitis in one and pyelonephritis in the other. In both of these cases pneumococci were isolated which failed to agglutinate in serums for types I to XX, and in the first case pneumococci of type III were present in addition. There was a case in which inguinal adenitis was present with type XI.

Empyema or pyothorax occurred in 5 cases. In a case in which type IV was found the condition was associated with streptococcic sepsis; in a case in which type IX was isolated, the condition was accompanied with staphylococcic sepsis, and in the remaining cases, with malignant neoplasms of the lung. Two of the neoplasms in cases in which types I and V were found, respectively, were bronchogenic carcinoma, and the third (in a case in which type III occurred) was metastatic from the colon. There were 2 cases in which bronchiectatic abscess was found with, respectively, type V and type VII.

There were 15 cases in which pneumococcic sepsis was observed. In 3 of the instances the condition was associated with vegetative endocarditis. In one of the latter cases, an infection due to type VIII probably originated in pyelonephritis and perinephritic abscess, and in a second case there was a pulmonary abscess with type I which may have been a postpneumonic complication. In 12 of the cases, no focus of infection could be found to account for the cultures of pneumococci that were obtained from the cardiac blood. In 2 cases there was pyelonephritis, associated, respectively, with a colon bacillus and a dysentery bacillus. It is of interest that in 9 of the 12 cases in which sepsis occurred without focal pneumococcic infection, there was definite evidence of damage to the liver, including: cirrhosis of the liver (a case in which type VIII was isolated), hemochromatosis (a case in which type V was found), icterus neonatorum (a case in which type II was present), congenital syphilitic cirrhosis of the liver (another case in which type II

was present), chronic passive congestion accompanying cardiac infarction and congestive failure (a case in which type I was found), primary or metastatic carcinoma (cases in which types I, III and XI were isolated) and fatty liver in a diabetic patient (with type V).

Other Conditions (Table 6).—This group included mostly cases in which cultures were made of sputum or of lung in the presence of pulmonary or mediastinal lesions that were probably not related to the types of pneumococci that were recovered. These were mainly cases of tuberculosis, pulmonary infarcts or hemorrhages, ruptured aortic aneurysm and nonpneumococcic sepsis. Pneumonia was not detected in the gross or in the microscopic sections of the lungs. The number of cases is small, but the distribution of types is not unlike that found in cases of atypical pneumonia.

COMMENT

We have been chiefly concerned in this paper with the bacteriology and the grosser anatomic findings in cases in which pathologic conditions were associated with specific types of pneumococci, classified with the aid of serums for the newer types of Cooper.[1] The available data in the literature dealing with this subject are meager. Many of the earlier observations, in addition to the fact that they are based on relatively small numbers of cases, are largely vitiated by the inadequacy of the identification of types. This is due not only to the failure to classify the strains formerly included in group IV but also, which is equally important, to the failure to identify properly the atypical strains of types II and III of the pneumococci. These are now recognized as important and frequently occurring types which can be definitely classified among the newer types. Types V and VI include most of the strains formerly identified as atypical strains of type II, and type VIII includes those regarded as atypical strains,[1] of type III. The importance of differentiating these groups of related types has been dealt with elsewhere.[6]

For the most part, we have dealt with a comparison of the findings in cases of lobar pneumonia and of atypical pneumonia and their relation to the types of pneumococci. Although the total number of cases presented is fairly large, considering the nature of the material, it is not adequate for a reasonably detailed comparison of the findings among any considerable number of types. This is due to the predominance of certain types in the cases of lobar pneumonia and the greater distribution of types in the cases of atypical pneumonia.

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6. (*a*) Finland, M., and Dowling, H. F.: Arch. Int. Med. **58**:598, 1936. (*b*) Finland, M., and Winkler, A. W.: J. Clin. Investigation **13**:79 and 97, 1934. (*c*) Finland and Sutliff.[4]

It is of interest to note that, although practically all types of pneumococci are represented among the clinical cases of lobar pneumonia both in this clinic [2] and at the Harlem Hospital,[7] there were twelve types not represented among the cases of lobar pneumonia which were

TABLE 6.—*Summary of Findings Among Eighteen Cases in Which Different Types of Pneumococci Were Cultured Without Evidence of Pneumonia or Focal Pneumococcic Infection*

Case	Sex	Age	Major Disease	Pneumococcus Type	Source	Other Organisms	Source
1	M	46	Mediastinitis (postoperative)	I	Sputum	Str. haemolyticus	Cardiac blood, pleura, pericardium, mediastinum
2	M	55	Pulmonary tuberculosis	I	Lung	Tubercle bacilli	Lung
3	M	48	Pulmonary tuberculosis	II	2 sputums	Str. haemolyticus and viridans; tubercle bacilli	Lungs
4	M	50	Pulmonary and intestinal tuberculosis, cirrhosis of liver	III, VII	Sputum	Tubercle bacilli	Lungs
5	M	38	Ruptured aortic aneurysm	III	Sputum	.....	.....
6	F	28	Septic abortion, peritonitis	III	Sputum	Staph. aureus, Str. viridans, Esch. coli	Blood, lung, pleura, pelvis
7	M	78	Scurvy, pulmonary hemorrhages	III	Sputum	.....	.....
8	M	45	Pulmonary and miliary tuberculosis	IV	Lung	Tubercle bacilli	Lungs
9	M	83	Prostatectomy, ascending pyelonephritis	IV	Throat	Str. viridans	Sputums
10	F	28	Chronic pelvic inflammation	VI	Sputum	.....	.....
11	M	50	Syphilitic aortitis, pulmonary infarcts	III, VIII	Different sputums	.....	.....
12	M	66	Pulmonary and miliary tuberculosis	VIII	Lung	Str. haemolyticus and viridans; tubercle bacilli	Lung, blood (Str. haemolyticus)
13	M	2	Laryngotracheobronchitis	IX	Lung	Str. haemolyticus	Cardiac blood, lung
14	M	41	Pulmonary tuberculosis	XVIII	Sputum	Staph. aureus, tubercle bacilli	Sputum, lung
15	F	36	Carcinoma of stomach, metastases to lungs	XXII	Sputum	Str. haemolyticus	Sputum
16	M	26	Rheumatic heart, pulmonary infarcts	XXIX	Sputum	Str. viridans	Lungs
17	M	52	Pulmonary and miliary tuberculosis	XXX	Lung	Tubercle bacilli	Lung
18	F	40	Dissecting aortic aneurysm	Negative I-XX*	Sputum	.....	.....

* The pneumococcus isolated failed to show agglutination with serums for types I to XX.

investigated post mortem. In the clinical series [2] there were included 117 cases of lobar pneumonia due to these types, with death occurring in 26. Such of the cases with these types as were examined at autopsy showed atypical pulmonary lesions; hence they were classified as cases

7. Bullowa, J. G. M., and Wilcox, C.: Arch. Int. Med. **59**:394, 1937.

of atypical pneumonia. In the cases without autopsy the classification was necessarily based on clinical observations, supplemented in almost all instances with roentgenograms. The discrepancy between the clinical and the anatomic observations suggests that in a considerable number of cases the clinical classification of lobar and bronchopneumonia is incorrect, even when aided with roentgenograms as they are ordinarily made for patients acutely ill with pneumonia.

Certain factors in the host have been pointed out as predisposing to atypical pneumonia with types which are otherwise predominantly associated with lobar pneumonia. Notable among these factors are: infancy and old age, preexisting chronic pulmonary diseases or other serious chronic diseases in the later stages, or acute episodes in the course of such illnesses, a variety of acute infections and finally injuries and operations. These factors have been brought out in previous clinical studies, in which their effects on the death rates were considered.[8] No further light has been thrown on this problem from the anatomic observations presented here. While it is possible that more detailed histologic studies of the lesions might yield further information in this direction, it is more likely that the anatomic differences are related to the general and local immunologic background of the host.

SUMMARY

We present the more important bacteriologic and anatomic findings in a series of 764 cases of infection with pneumococci in which autopsies were made and the cultures of pneumococci were typed with the aid of specific serums for types I to XXXII (Cooper). The data concerning the 684 cases of pneumonia included in this study indicate that, in a general way, a relationship exists between the type of pneumococcus, the character of the pulmonary lesion resulting from the infection and the associated complications.

Lobar pneumonia as defined here was limited within a smaller number of types of pneumococci than was atypical pneumonia. In cases of lobar pneumonia, types I, III and II were most frequent, and types V, VII, VIII and IV ranked next, in that order. These seven types together accounted for 90 per cent of the cases, and the remaining 10 per cent included eleven other types. In cases of atypical pneumonia, type III was by far the most frequent, with types VIII, V, I, X, VII, VI, XX, XVIII and II following in that order. These ten types accounted for 71 per cent of the cases, the remainder including sixteen other types. The cases of pneumonia associated with types I and II were most regularly cases of lobar pneumonia (more than 80 per cent), whereas somewhat over a third of the cases asso-

8. Finland.[2] Finland and Sutliff.[4] Finland and Dowling.[6a]

ciated with types III, IV, V and VII and considerably over half of those associated with the remaining types were cases of atypical pneumonia.

Focal purulent complications were more frequent among cases of lobar than among cases of atypical pneumonia due to the same type of pneumococci. Empyema was most common in cases due to type I and abscess (necrosis) of the lung in cases due to type III. Pneumonia occurring in the course of underlying chronic diseases of the respiratory tract, during serious acute diseases or during acute episodes of chronic illnesses was more commonly atypical and associated with the less frequent types of pneumococci; pneumonia complicating pregnancy and the puerperium was, in the predominant number of cases, lobar and due to the more common types of pneumococci.

Cultures of pneumococci from blood obtained during life or at autopsy were considerably more frequent in cases of lobar pneumonia than in those of atypical pneumonia due to the same types. Organisms other than the respective predominant types of pneumococci were cultured in a large proportion of the cases of pneumonia. Streptococci, staphylococci, influenza bacilli, Friedländer's bacilli and heterologous types of pneumococci were the most common. They were found with similar frequency in the groups of cases due to the respective types of pneumococci and were obtained in about a third of the cases of lobar and half of those of atypical pneumonia. Heterologous pneumococci and Friedländer's bacilli were the only organisms found with greater frequency in the cases of lobar pneumonia.

It is to be emphasized that the data presented are based on cases in which autopsies were made. In their application to the general problem of pneumococcic infections, due allowance must be made for the factors influencing the fatality rates and for the limitations of clinical methods.

EPITHELIAL METAPLASIA OF THE THYROID GLAND

WITH SPECIAL REFERENCE TO THE HISTOGENESIS OF SQUAMOUS CELL CARCINOMA OF THE THYROID GLAND

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In the modern literature the tendency prevails to consider the islands of squamous epithelium which are occasionally found in the thyroid gland as vestiges of embryonic structures. This explanation may satisfy in the very rare instance in which the gland of a newborn infant or small child contains the abnormal epithelial inclusions (Koeppel [1]; Getzowa [2]). It will meet with objections, however, when it comes to account for the occurrence of squamous epithelium in thyroid glands which are the site of severe pathologic changes. Several years ago, Meeker [3] described in Riedel's goiter peculiar groups of large polygonal cells with vesicular nuclei which, in accordance with Getzowa, she was inclined to link to the ultimobranchial body. Her explanation created interest since it seemed to cast light on the obscure pathogenesis of the iron-hard goiter. One may recall, however, that, in the human being, the ultimobranchial body, which develops from a caudoventral extension of the fourth pharyngeal pouch, is a very transient structure. After becoming included in the lateral wings of the thyroid gland at the 25 mm. stage of the embryo, it undergoes reticulation and degeneration, and one may very well doubt whether it contributes to the thyroid parenchyma (Kingsbury [4]; Watzka [5]). Wegelin [6] assumed that the islands of squamous epithelium described by Koeppel in his study of thyroid glands from various parts of Germany may be derived from the thyroglossal duct. On the other hand, Wegelin referred to epithelial metaplasia in three cases of adenomatous goiter containing squamous epithelium, in one case with rudimentary hornification.

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1. Koeppel, F. C.: Beitr. z. path. Anat. u. z. allg. Path. **49**:579, 1910.
2. Getzowa, S.: Virchows Arch. f. path. Anat. **188**:181, 1907; **205**:208, 1911.
3. Meeker, L. H.: Am. J. Path. **1**:57, 1925.
4. Kingsbury, B. F.: Anat. Rec. **61**:155, 1935.
5. Watzka, M.: Ztschr. f. mikr.-anat. Forsch. **34**:485, 1933.
6. Wegelin, C.: Schilddrüse, in Henke, F., and Lubarsch, O.: Handbuch der speziellen pathologischen Anatomie und Histologie, Berlin, Julius Springer, 1926, vol. 8.

Boyden, Coller and Bugher [7] and Risak [8] spoke of metaplasia of the thyroid epithelium to squamous epithelium in Riedel's goiter.

When studying the effect of subcutaneous injections of ortho-amido-azotoluene, the principal constituent of scarlet red, on the thyroid gland of the guinea-pig, Joshida [9] observed disintegration of the follicles and proliferation of connective tissue. In addition, he also found proliferation of the follicular epithelium, which assumed the properties of squamous epithelium with hornification. Heep [10] and Brass [11] fed rats and mice ortho-amido-azotoluene. Heep encountered but once a duct in the thyroid gland lined by squamous epithelium, which he considered as a residue of the branchial cleft and not as a result of epithelial metaplasia. Among twenty-nine mice fed ortho-amido-azotoluene Brass found squamous epithelium in the thyroid gland twice but did not venture to decide whether he dealt with metaplasia or with malformation.[11a]

It is the purpose of this paper to show that under certain pathologic conditions metaplasia of the follicular epithelium of the thyroid gland to squamous epithelium occurs and that this epithelial metaplasia offers the most logical explanation for the histogenesis of the squamous cell carcinoma of the thyroid gland.

CASES OF METAPLASIA OF THE FOLLICULAR EPITHELIUM OF THE THYROID GLAND IN THE PRESENCE OF SCLEROSIS OF THE GLAND

In three cases I have encountered metaplasia of the cuboid epithelium of the follicles into squamous epithelium, in sclerosis of the thyroid gland. Since two of these cases have been described in detail elsewhere, those changes only will be presented which are connected with the metaplasia. Both cases concerned elderly women whose outstanding clinical finding was marked anemia. The thyroid gland had been replaced by dense connective tissue, and besides atrophy of the follicles regeneration was evident. The regenerative epithelial proliferation led to the formation of small circumscribed nests of cells. Some of these nests were composed of dark-nucleated small cuboid cells, while others were formed by large elongated and polygonal cells with ample homogeneous cytoplasm, sharply defined cell outlines and vesicular nuclei. Here and there fine interepithelial bridges could be seen. The larger nests consisted of a peripheral layer of cuboid cells and a central zone of polygonal cells. The cells of the central portion occasionally showed hyalinization of the cytoplasm. A few of the cell nests were

7. Boyden, A. M.; Coller, F. A., and Bugher, J. C.: West. J. Surg. **43**:547, 1931.

8. Risak, in discussion on Slany.[23]

9. Joshida, T.: Virchows Arch. f. path. Anat. **283**:29, 1932.

10. Heep, W.: Frankfurt. Ztschr. f. Path. **50**:48, 1936.

11. Brass, K. A.: Frankfurt. Ztschr. f. Path **50**:63, 1936.

11a. C. A. Helwig (Am. J. Cancer **23**:550, 1935) described metaplastic squamous epithelium in a thyroid adenoma of a rat which had been kept on a calcium-rich diet.

surrounded by lymphocytic infiltrations. In sections stained with silver, diamond-black fibrils encircled the groups of squamous epithelial cells in the same manner as they encircled the follicles of the thyroid gland (fig.1 *A*).

In the third case, that of a woman aged 54 years who had died from acute atrophy of the liver, the thyroid gland weighed 25 Gm. It was firm and light gray-tan, with islands of dark red tissue, and contained a small nodule rich in colloid. Throughout the gland there was much atrophy of the follicles, also marked proliferation of the interstitial tissue with round cell infiltrations. The connective tissue enclosed follicles in different stages of atrophy and groups of squamous epithelial cells as described in the foregoing paragraph.

A CASE OF METAPLASIA OF THE FOLLICULAR EPITHELIUM OF THE THYROID GLAND IN THE PRESENCE OF A METASTATIC ABSCESS

Ulcerative endocarditis of the aortic valve caused the death of a white woman 42 years of age. In the clinical history no symptoms were recorded that were referable to the thyroid gland. Suppurative meningitis developed shortly before death. Cultures from the meningeal exudate and the spleen showed hemolytic streptococci.

Description of Thyroid Gland.—The thyroid gland weighed 30 Gm. It was moderately firm, granular and light brown and contained many small nodules, which were rich in colloid. In the upper pole of the right lobe there was a cavity which measured 10 mm. in diameter and was filled with thick, light yellow pus. Smears taken from this pus revealed many long chains of gram-positive streptococci.

Histologic Examination.—The gland was found to be composed of medium-sized follicles, the majority of which were in the resting stage. There was slight epithelial proliferation. The abscess involved a node which was well separated from the surrounding tissue. This node was made up of large follicles, which often fused together and contained thin colloid. In focal areas the stroma was increased and hyalinized and contained needles of fatty acid with foreign body giant cells and deposits of blood pigment. The abscess was lined by a membrane of vascular granulation tissue, densely infiltrated by lymphocytes, plasma cells, histiocytes and leukocytes. The cavity was filled by pus cells and necrotic débris.

Near the abscess several apparently isolated islands of squamous epithelium were found. Some of the islands revealed evidences of central keratinization. On serial section it was found that these islands were connected with follicles by narrow channels, which were lined by a single layer of flattened epithelial cells (fig. 1*B*). The follicles connected with the islands of squamous epithelium showed a low cuboid epithelium that, in places, was interrupted by squamous epithelium. The latter consisted of several layers of flattened elongated cells, resting on a basal layer of dark-nucleated cuboid cells.

COMMENT ON FOREGOING FOUR CASES

In the cases which I have described there was no indication that the squamous epithelial cells in the thyroid gland may have been derived from embryonic structures such as the thyroglossal duct, the ultimobranchial bodies or the branchial clefts. I believe that the histologic

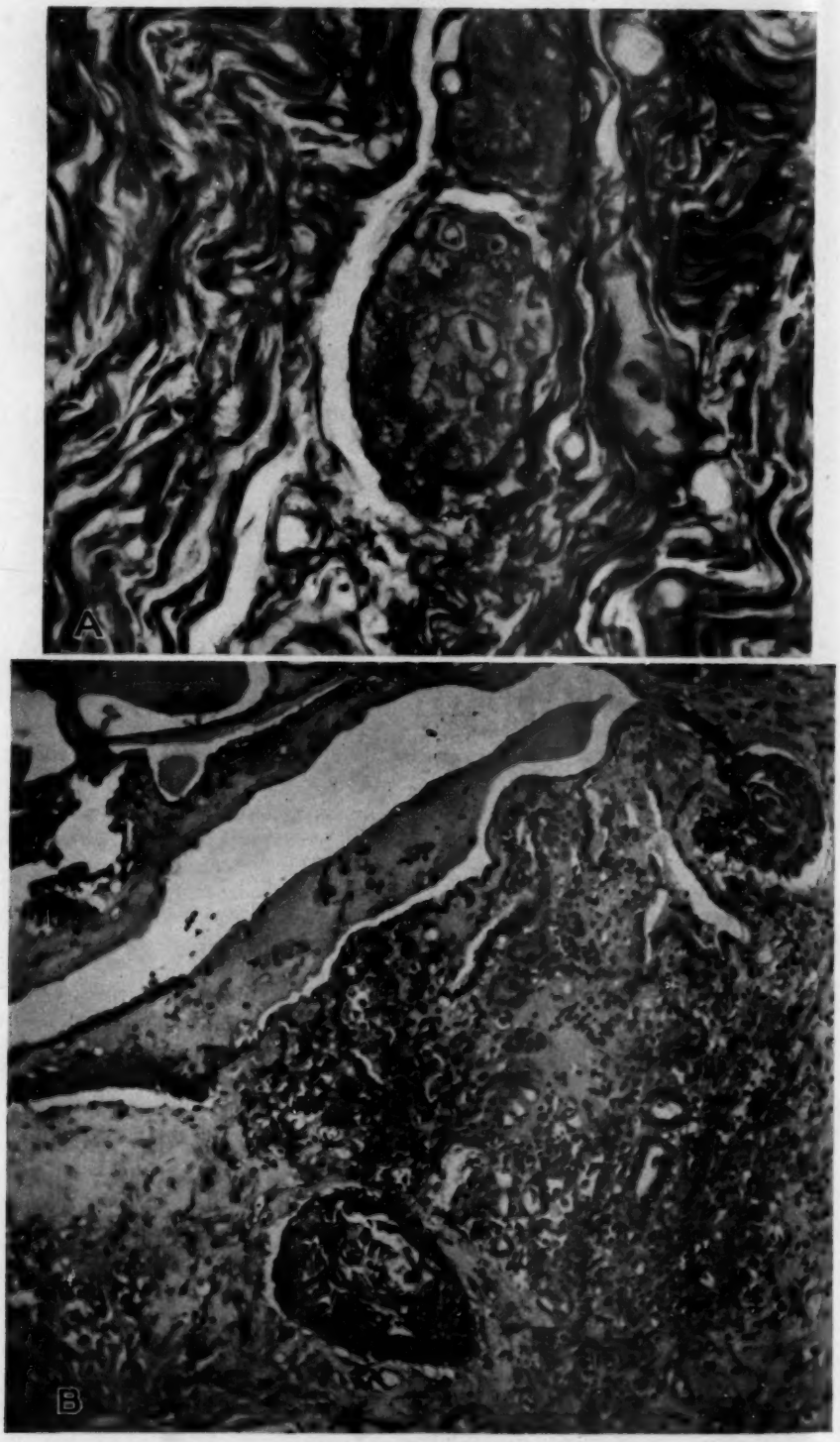

Fig. 1.—*A*, island of squamous epithelium in sclerotic thyroid tissue; silver impregnation; magnification, × 600. *B*, islands of squamous epithelium near an abscess in thyroid tissue; hemalum-eosin; magnification, × 150. Note the island connected with a flattened follicle.

observations leave no doubt as to the metaplastic origin of these cells. The sclerotic thyroid glands revealed evidences of epithelial regeneration, which seemed to start from incompletely involuted follicles. At first, the regenerated epithelial cells appeared undifferentiated. They were small, cuboid and dark-nucleated, and they were aggregated to small solid nests. When the nests grew larger, the cells increased in size and assumed a polyhedral shape, and the ample cytoplasm became condensed near the cell membrane. The nuclei were vesicular. Here and there, epithelial bridges became discernible, and the centrally located cells underwent hyalinization, with pyknosis of the nuclei. In the abscessed thyroid gland the change in the character of the follicular epithelium was confined to the vicinity of the abscess. Islands of squamous epithelium were found inserted in the follicular lining, and there was a branching-off of solid buds of squamous epithelial cells, which often showed definite epithelial fibers.

In order that metaplasia may take place, it is necessary that old cells degenerate and that young and not yet fully differentiated cells proliferate and meet with changed environmental conditions (Lubarsch [12]). If these requirements are fulfilled in the thyroid gland the follicular epithelium may change its character as epithelial cells do in other organs. If the proliferation of the metaplastic epithelium assumes the properties of autochthonous growth, neoplasms will result which, at the first glance, suggest a dysontogenic origin. Krompecher's basal cell theory does not apply to the thyroid gland since the follicular lining has no basal cells (Wegelin).

THREE CASES OF SQUAMOUS CELL CARCINOMA OF THE THYROID GLAND

Squamous cell carcinoma is the rarest type of cancer of the thyroid gland, and the majority of statistical reports on malignant growth of this gland quote only single observations (Clute and Smith [13]; Shallow, Lemmon and Saleeby [14]; Smith, Pool and Olcott [15]; Clute and Warren,[16] and others). Clute and Warren [16] included it in their group III, which comprises the hopelessly malignant growths, terminating in death usually within less than a year after the onset of the first symptoms. Roentgen ray and radium treatment seem to be of little avail. It is a disease of advanced age, occurring more frequently in males

12. Lubarsch, O.: Deutsche Ztschr. f. Chir. **227**:48, 1930.
13. Clute, H. M., and Smith, L. W.: Arch. Surg. **18**:1, 1929.
14. Shallow, T. A.; Lemmon, W. T., and Saleeby, E.: Ann. Surg. **101**:1190, 1935.
15. Smith, W. L.; Pool, E. H., and Olcott, C. T.: Am. J. Cancer **20**:1, 1934.
16. Clute, H. M., and Warren, S.: Surg., Gynec. & Obst. **60**:861, 1935.

than in females. Schmidtmann [17] reported a squamous cell carcinoma of the thyroid gland in a boy 10 years of age, which she traced to the branchial arches. The tumor affects glands which are the site of goitrous changes and forms very firm, pearly gray nodes with infiltrative growth. Histologically, one finds all degrees of differentiation up to well developed prickle cells with horn pearls. The stroma is often densely infiltrated by inflammatory cells. Metastases occur along the lymph stream.

If microscopic examination of a specimen from the region of the thyroid gland discloses squamous cell carcinoma, one is inclined to consider first a tumor that has originated outside the gland and is invading it secondarily. This holds particularly true if the tumor is located laterally, suggesting a branchiogenic carcinoma with extension to the thyroid gland. In other instances a carcinoma of the larynx, pharynx or esophagus may spread to the thyroid gland. Hence, only those cases will stand criticism in which the diagnoses are proved at autopsy. I have collected three cases of squamous cell carcinoma of the thyroid gland which came to autopsy and which, in connection with the discussion of the metaplasia of the follicular epithelium of the thyroid gland, may be presented briefly.

Case 1.—Three and a half months prior to admission to the hospital a white man 58 years of age noticed a firm lump on the left side of the neck, which grew very rapidly and soon reached the size of an orange. The mass caused much pressure deep in the throat and severe pain on swallowing. On admission it was found to move with the thyroid gland and not to be adherent to the overlying skin. Anterior to both sternocleidomastoid muscles, small firm lymph nodes could be felt. A specimen of tissue was taken for biopsy, and when the report was returned that the mass was a squamous cell carcinoma, most of the left lobe of the thyroid gland was removed with the aid of electrosurgery. However, one month later the patient was readmitted to the hospital, showing extensive recurrence. The left internal jugular vein was ligated, and nasal feeding was instituted. The patient grew rapidly weaker and died four weeks after the second admission.

Description of Surgical Specimen.—The removed portion of the thyroid gland weighed about 35 Gm. It consisted of very firm gray-white tissue with streaks of softer purple-brown and finely granular tissue, and multiple nodes averaging 20 mm. in diameter. Some of these nodes were stony hard.

Microscopic Description.—Most of the thyroid tissue had been replaced by variously sized alveoli, which were composed of large slightly flattened polygonal cells with oval nuclei containing a net of small chromatin granules and distinct nucleoli. At the periphery of the alveoli the cells were often cuboid. There was a moderate amount of stroma, which in many places was densely infiltrated by polymorphonuclear leukocytes. Where the stroma was more abundant the tumor cells formed cords and small nests, and in the center of the nests the cells arranged themselves concentrically and became keratinized (fig. 2). The stony nodules were composed of dense hyalinized connective tissue with much calcifica-

17. Schmidtmann, M.: Virchows Arch. f. path. Anat. **226**:100, 1919

tion. In the periphery of the nodes, encased in the dense tissue, single flattened follicles were found. An occasional follicle was seen which was lined partially by low cuboid epithelial cells and partially by up to five layers of flattened cells with relatively large, hyperchromatic nuclei. In one place, near a node, a small papillary adenoma was found.

Abstract of Autopsy Reports.—Autopsy showed: a recurrent squamous cell carcinoma of the left lobe of the thyroid gland with metastases in a scar from Kocher's operation, in the right lobe of the thyroid gland and in the deep cervical lymph nodes of both sides; diffuse carcinomatous infiltration of the soft structures of the neck with constriction of the esophagus, trachea and left recurrent

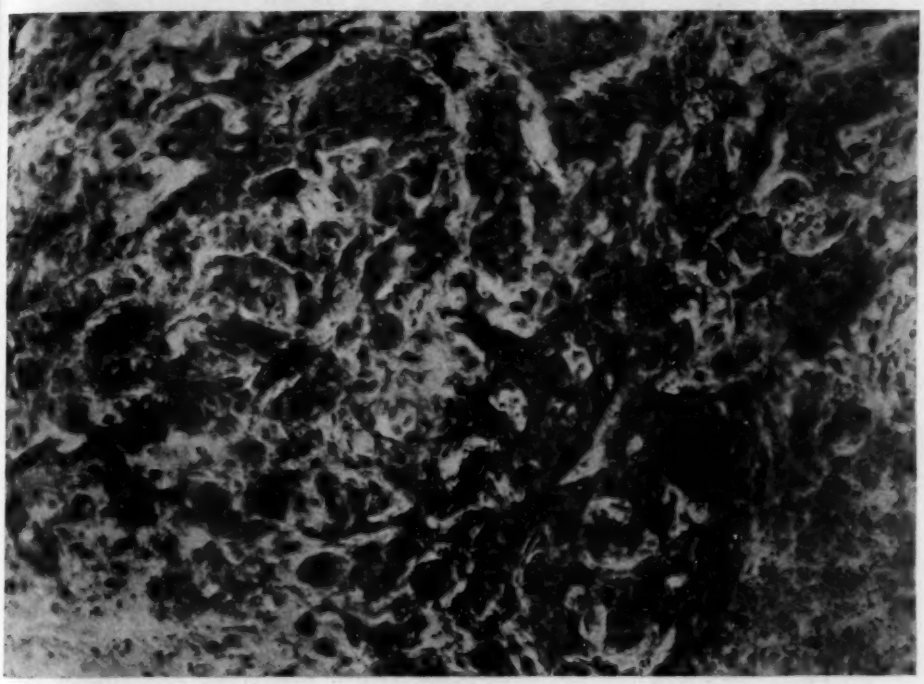

Fig. 2.—Squamous cell carcinoma of the thyroid gland (case 1); hemalum-eosin; magnification, × 150.

laryngeal nerve; tumor invasion of both internal jugular veins with secondary thrombosis; metastases in the apex of the right ventricle of the heart and in the pleura of both lungs.

Abstract of Histologic Report.—The tumor masses of the neck as well as the metastases were composed of large polygonal cells with ample clear cytoplasm and oval nuclei showing a sharply defined granular chromatin net and distinct nucleoli. Everywhere the infiltration of the stroma by polymorphonuclear leukocytes was a striking feature.

CASE 2.—A white man aged 48 had been troubled with pains in the lower part of the spine for five months. Later, his neck started to swell, and he experienced difficulty in walking. On admission to the hospital he presented a firm mass

on the left side of the neck, apparently located within the thyroid gland. Roentgen examination revealed a destructive process in the third and fourth dorsal vertebrae. The patient died soon after admission.

Abstract of Autopsy Report.—The observations at autopsy were: a squamous cell carcinoma of the left lobe of the thyroid gland with metastases in the left cervical, axillary, tracheal and peribronchial lymph nodes, in the liver and in the bodies of the third and fourth dorsal vertebrae; slight compression of the spinal cord; catarrhal cystitis and pyelitis; fibroplastic deformity of the mitral and aortic valves; cholelithiasis.

Description of Thyroid Gland.—The gland weighed 48 Gm. and the left lobe was about double the size of the right. The right lobe was moderately firm and

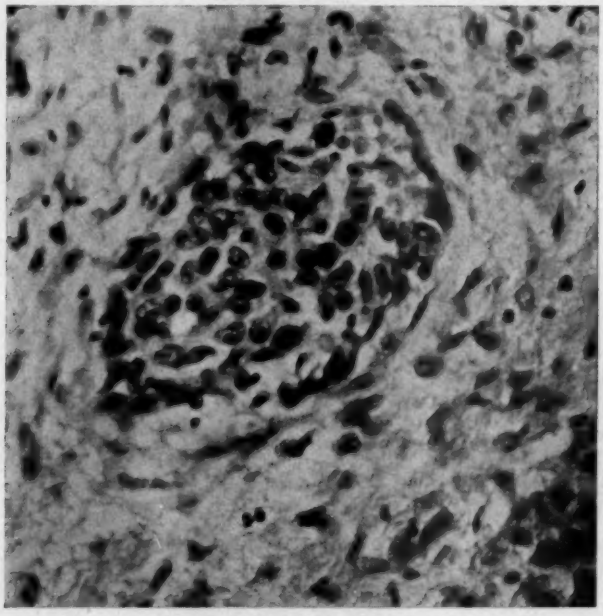

Fig. 3.—Squamous cell carcinoma of the thyroid gland (case 3); hemalum-eosin; magnification, × 300.

presented a uniformly granular and light purple-brown appearance. The left lobe contained a single node, 22 mm. in diameter, firm and of a light gray color. On the sectioned surface of the node many yellow lines and patches were seen. The deep cervical lymph nodes averaged 12 mm. in diameter.

Abstract of Histologic Report.—The thyroid gland was composed of large follicles in the resting stage. The lining cells of the follicles were uniformly low cuboid. The node in the left lobe was made up of alveoli of polygonal cells with ample homogeneous cytoplasm and oval nuclei that were moderately rich in chromatin. In the center of the larger alveoli the cells were often smaller and more compact, with the tendency to form concentric layers. The alveoli were embedded in much dense connective tissue. The metastases resembled the primary tumor, except the metastases in the spine, in which the cells were decidedly smaller, spindle shaped and very dark nucleated.

CASE 3.—The first symptom of the patient, a white man 58 years of age, was the appearance of a lump on the head, which within two months grew to the size of an apple. Associated with the growth on the head were weakness, loss of appetite and loss of weight. He regurgitated everything he ate or drank and finally lost his voice so that he could speak only in a whisper. He coughed frequently, and the sputum was often bloody. On admission he showed, underneath the scalp over the left parietal bone, a moderately firm node 8 cm. in diameter. The thyroid gland was enlarged, firm and nodular, and the lymph nodes on both sides of the neck were enlarged. The patient died three days after admission.

Abstract of Autopsy Report.—Autopsy demonstrated: a squamous cell carcinoma of the left lobe of the thyroid gland with metastases in the peritracheal, periesophageal, posterior mediastinal, left supraclavicular and bifurcation lymph nodes, in the mesentery and in the left parietal bone; marked compression of the trachea and esophagus by the metastases in the lymph nodes and invasion of the lower half of the esophagus; destruction of the left recurrent laryngeal nerve and invasion of the left internal jugular vein; moderate hypertrophy of the heart; chronic emphysema of the lungs; chronic suppurative cholecystitis; cholelithiasis; thrombosis of the prostatic veins.

Description of Thyroid Gland.—The gland weighed 49 Gm. The left lobe measured 7 by 4 by 3.5 cm., and the right lobe, 6 by 5 by 3 cm. In both lobes were multiple nodes. The largest of the nodes was found in the lower pole of the left lobe and measured 45 mm. in diameter. This node consisted of purple-brown tissue with, up to 35 mm., firm gray-white homogeneous areas.

Abstract of Histologic Report.—The thyroid gland was composed of medium-sized and large follicles, which were lined by low cuboid epithelial cells and were filled with thin and vacuolated colloid. In the largest node there were numerous nests, cords and larger alveoli of large polygonal or elongated cells with well defined cell borders and oval nuclei (fig. 3). The central portions of the larger alveoli were necrotic. The small veins were often filled by tumor cells. The tumor tissue occupied the space between the follicles, often compressing and invading them. The stroma was increased. In the metastases the squamous cell character of the tumor tissue was well pronounced.

COMMENT ON FOREGOING GROUP OF THREE CASES

Most of the authors who have dwelt on the histogenesis of squamous cell carcinoma of the thyroid gland traced the tumor to residues of the thyroglossal duct (Clute and Warren [16]; Ewing [18]; Kaufmann [19]; Roeder [20]; Rubenstone and Meronze [21]; Smith, Pool and Olcott [15]; Wegelin,[6] and others). Kocher [22] stated that the squamous cell carcinoma affects most frequently the left lobe of the thyroid gland, in

18. Ewing, J.: Neoplastic Diseases, Philadelphia, W. B. Saunders Company, 1928.

19. Kaufmann, E.: Lehrbuch der speziellen pathologischen Anatomie, Berlin, W. D. de Gruyter & Co., 1922.

20. Roeder, C. A.: Ann. Surg. **73**:23, 1921.

21. Rubenstone, A. J., and Meronze, D. R.: Arch. Path. **14**:587, 1932.

22. Kocher, T.: Deutsche Ztschr. f. Chir. **91**:197, 1908.

which remnants of the thyroglossal duct are apt to occur. Slany [23] considered an adenocancroid of the thyroid gland as dysontogenic.

As in other instances in which tumors occurring in advanced age are linked to fetal inclusions, it is difficult to conceive that nests of fetal cells should have remained dormant over such a long period of time before starting to proliferate. A few investigators, therefore, have admitted that in some cases a squamous cell carcinoma of the thyroid gland may develop from metaplastic follicular epithelium (Herrenschmidt [24]; Bérard and Dunet [25]; Masson [26]; Wegelin [6]). At the time the tumor comes under observation the changes usually have advanced too far to allow any conclusions as to preexistent lesions from which the tumor may have originated. In this connection case 1 is of significance since it shows, in addition to the squamous cell carcinoma, metaplasia of the follicular epithelium to squamous epithelium in the peripheries of petrified nodes. This observation strongly suggests that epithelial metaplasia rather than residues of the thyroglossal duct is the source of squamous cell carcinomas of the thyroid gland.

SUMMARY

Four instances of metaplasia of the follicular epithelium of the thyroid gland are described in which this alteration has been associated with sclerosing and inflammatory changes in the gland. The epithelial metaplasia is considered as the source of the rare squamous cell carcinoma of the thyroid gland, of which three additional cases are reported.

23. Slany: Centralbl. f. allg. Path. u. path. Anat. **66**:257, 1936.

24. Herrenschmidt, A.: Carcinomes pavimenteux du corps thyroïde, Thèse de Paris, no. 225, 1904.

25. Bérard, L., and Dunet, C.: Cancer thyroïdien, Paris, Gaston Doin & Cie, 1924.

26. Masson, cited by Wegelin.[6]

AMELOBLASTOMA

A SURVEY OF THREE HUNDRED AND SEVENTY-NINE CASES FROM THE LITERATURE *

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The ameloblastoma is a tumor with characteristics highly suggestive of an origin from odontogenic tissue. The first complete description is usually credited to Falkson (1879), although a number of suggestive cystic tumors of the jaw were previously reported. Since these early reports a great number of cases have been reported in the literature, each author pointing out the rarity of the growth, and few attempting to survey the previous reports. The literature was reviewed in connection with a histologic study, and because of the surprising number of references available, this survey was undertaken in an effort to consolidate opinion regarding the occurrence, malignancy and cause of the ameloblastoma.

CLASSIFICATION, DEFINITION AND SYNONYMS

The classification of tumors in general and oral tumors in particular is confusing. As Ewing has pointed out, there are three obvious plans for classifying neoplasms: the histologic, the regional and the etiologic. While the latter is probably the most desirable, the scanty knowledge embraced in this phase of oncology will permit the use of such a plan only in modifying a histologic index.

A number of classifications have been suggested for oral tumors, including those suggested by the Committee on Odontomes of the British Dental Association, Bland-Sutton, Wohl, Ivy and Churchill, Churchill and Geschickter. Of these classifications, it is felt that Churchill's [1] is the most acceptable on the basis of correctness of nomenclature and completeness. He classified the oral tumors under various headings and subheadings. Under "Blastomata," tumors derived from one type of

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* A bibliography will be appended to this article in the reprints. When a contribution on the subject is cited in the text the date is given only if there has been more than one contribution by the author named. A letter added to the date distinguishes between contributions published in the same year.

† Rockefeller Fellow in Dentistry.

1. Churchill, H. R.: Beiträge zur Klassifizierung der Tumoren des Mundes, und seiner Nebenorgan, Inaug. Dissert., Rostock, Carl Hinstorffs Hofbuchdruckerei, 1932.

tissue, he included the division "epidermal blastomata," with a subgroup for neoplasms derived from the odontogenic apparatus. Under the latter grouping he included "ameloblastomata." These are subdivided into the usual clinical types: solid, cystic and, a unique class, melanotic. Both benign and malignant variations are considered. Schematically, the classification may be represented in part:

Blastomas

Tumors of the epidermis and those from its degenerated tissues and tumors of the squamocellular mucosa

Tumors from the odontogenic apparatus

Benign			Malignant
Ameloblastoma	Solid	Dentocystic tumors	Carcinoma ameloblasticum
with cornification	Cystic		Sarcoma ameloblasticum [2]
without cornification	Melanotic		
Dentigerous cyst			
Simple, compound			
Eruption, heterotropic			
Dentoperiosteal cyst			

The ameloblastoma may be defined as an epithelial tumor arising from the odontogenic apparatus or from cells with a potentiality for forming tissues of the enamel organ. Its structure resembles histologically that seen in certain developmental stages of the enamel organ. It is usually unicentric, nonfunctional, intermittent in growth, anatomically benign and clinically persistent. It is most commonly found in the mandible or in the maxilla, but in many cases it has been discovered in the hypophysis, and in at least seven cases, in the tibia.

A great many names have been applied to this growth, but the term "ameloblastoma," suggested by Ivy and Churchill, seems to be the most descriptive. This name is derived by combining "ameloblast," designating the enamel forming cell, with "blastoma," designating a tumor of one type of tissue. As Galippe pointed out, the more common term, "adamantinoma," indicates a growth composed of enamel and consequently is unsuited to this tumor, which never forms adamantine tissue. Lederer and Riethmueller used a variant of this term, "adamantoma," to describe an enamel pearl. The term "ameloblastoma" has been accepted by Frazier and Alpers to describe the corresponding hypophysial tumor. In addition to the names already mentioned, there are at least twenty additional synonyms in the English language and a large number in German, French and other languages (Robinson, 1936).

2. Not given by Churchill in the Rostock dissertation, but given to the author in a personal communication in June 1934.

GENERAL REVIEW

Although Falkson is generally credited with the first complete description of the ameloblastoma, several cystic tumors of the jaw had been reported before he described the neoplasm. Guzack, in an account of his operations for resection of the mandible, described 7 lesions of the lower jaw, at least 3 of which (cases 1, 6 and 7) are highly suggestive of ameloblastoma, but one hesitates to list these definitely as such because of the somewhat incomplete pathologic descriptions. Broca studied tumors of this type and concluded that they represented an overgrowth of the dental germ. Heath studied a number of the tumors and also observed a sarcomatous cystic tumor of the jaw. Eve studied the growths rather thoroughly, pointed out their homology with the enamel organ, noted that they frequently followed injury, irritation from decayed teeth and inflammation, and observed that half of them occurred between the twentieth and fortieth years of life. Malassez published two extensive papers on epithelial débris and postulated a connection between epithelial remnants about the roots of teeth and the ameloblastoma. Simmons reported 14 cases that he had observed. Kronfeld described the transition from the solid to the cystic in a series of cases. McFarland and Patterson reviewed the literature, collecting reports of 166 cases of ameloblastoma of the jaw and of 26 cases of ameloblastoma of the pituitary. Kegel reported 35 cases and reviewed the literature. He found the corrected ratio of occurrence between the black and white races to be 11:1. Kotanyi reported 26 cases and estimated that 8.89 per cent of all cases of benign tumor of the jaw were instances of ameloblastoma, basing this conclusion on the lesions encountered in Pichler's clinic. Darlington reported 1,000 cases of dental tumor, of which 12 were instances of ameloblastoma. McGregor (1935) reported 11 cases and made a special study of the cases of malignant tumor of this type recorded in the literature.

STATISTICAL SURVEY

In making this survey difficulty was encountered in deciding which reported cases of oral tumor should be included as instances of ameloblastoma. It is apparent that the author of a report is in a better position for judging the nature of his case than the critical reviewer. For this reason any case in which it is apparent that the author believed himself to be dealing with a tumor fitting the definition of an ameloblastoma has been included. Only cases of ameloblastoma appearing about the maxilla or the mandible have been considered, to the exclusion of those in which the growth involved the pituitary gland or the tibia.

The first fact that becomes evident to a reviewer of the literature on this subject is that ameloblastomas are not as rare as is generally believed. At least 379 cases have been reported in various English,

German, French, Italian and Scandinavian periodicals. This appears to be the greatest number of cases collected to date, being approached only by McFarland and Patterson's 166 cases.[2a]

Complete study of many of these cases was handicapped by incompleteness or, in a few instances, inaccessibility of the reports. In 311 cases, however, the sex is stated, 54.3 per cent (169 cases) appearing in females. In very few reports is any mention made of the patient's race. If it is assumed that those reports in which there is no statement about the race concern Caucasians, the 30 Negroes, 2 Chinese and 2 East Indians mentioned represent only a small percentage. While the foregoing assumption is hardly justified, it seems to indicate that Kegel's ratio of 11:1 in favor of the Negro race is likewise unjustified. In the 248 cases in which the age of the patient is given, the average is 37.6 years; in one instance an ameloblastoma appeared at birth; the oldest patient was 76. The data on age are not significant, as the figure given in each instance represents merely the age of the patient at the time of presentation, operation or discharge, varying from author to author. By subtracting from the age stated in the report the so-called duration, one arrives at the age of the patient when the ameloblastoma was discovered, which approximates the age of onset. While it cannot be claimed that this is the age at which the growth actually began, it is the age at which either objective or subjective symptoms were recognized, a figure of some clinical importance. This age at discovery could be computed in 222 instances and was found to average 30.1 years. The distribution of these cases is given in chart 1. When these cases are plotted in ten year periods, a fairly smooth curve is obtained. It is found that 70 per cent of the tumors were discovered between the tenth and thirty-fifth years. The figures for the age at discovery are probably high because of the slowness and painlessness of growth which characterize the ameloblastoma and which may result in failure to discover the growth for months or years. Similarly the figures for duration are probably lower than the actual. This duration or, more exactly, lapse of time between the discovery and the age as stated in the report was found for 232 cases and averaged 8.5 years. The range of the so-called duration is given in chart 2. A study of chart 2 shows that these are probably approximations, because the fifth, tenth, fifteenth, twentieth and twenty-fifth years are relatively greater. A plot of five year periods shows that most of the durations fall in the early periods as might well be expected. The long duration, up to fifty-three years, is worthy of note in consideration of the degree of invasiveness of the growth. Of 295 cases, 83.7 per cent (247 cases) were in the mandible alone, and the remaining 16.3 per cent (48 cases), in the maxilla. One instance is recorded of a

2a. A table summarizing each of the 379 cases reviewed is included in the reprints.

growth involving both the maxilla and the mandible. Twice the ameloblastoma occurred atypically about the face; once on the upper lip (Wohl) and once in the orbit (Suker). The size in the cases reviewed varied from that of a small piece of shot, as in one of the cases listed, to that of the tumor reported by Bryk, weighing 1.5 Kg. and hanging from the mandible to the level of the second rib.

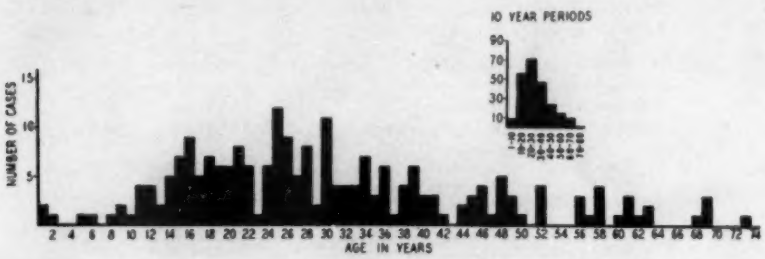

Chart 1.—The age at onset (age of patient as given in report minus time elapsed since discovery of tumor).

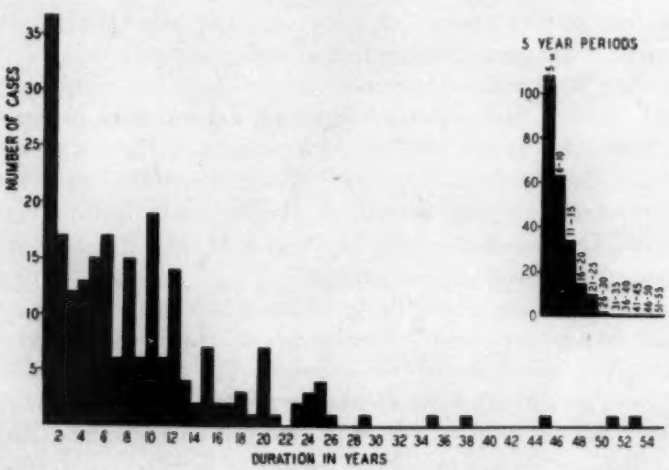

Chart 2.—The duration of ameloblastoma (age of patient as given in report minus age at time of discovery of tumor). The graph is based on 236 cases.

Most of these ameloblastomas were in cystic form at the time of operation, 57.5 per cent of the 219 instances in which this feature was reported being cystic, 24.2 per cent both cystic and solid, and 19.1 per cent solid. Fourteen of the reported tumors contained unerupted teeth. To these ameloblastomas containing teeth Kaufman (cited by Thoma) has given the name "odonto-adamantinoma." Although most authorities are agreed that the ameloblastoma never forms enamel, 3 enamel-containing tumors have been reported under this classification (Bernays, Chibert, d'Aunoy and Zoeller). Ghosh reported an instance in which

a substance suggestive of the matrix of enamel was apparent within the cysts. Formation of enamel pearls, keratinization or hornification was reported for 16 of these tumors. The case of melanotic ameloblastoma containing a calcified mass reported by Mummery and Pitts is apparently unique.

These statistical findings can be summarized in tabular form:

		Cases
Total number of cases		349
Sex (311 cases)		
Male	45.7 per cent	142
Female	54.3 per cent	169
Average age at time of report	37.6 years	248
Average duration of tumor	8.5 years	232
Average age at time of discovery	30.1 years	222
Site of growth (293 cases)		
Mandible	83.7 per cent	247
Maxilla	16.3 per cent	48
Structural characteristics (219 cases)		
Cystic	57.5 per cent	124
Cystic and solid	24.2 per cent	53
Solid	19.1 per cent	42

MALIGNANCY

The malignancy of these tumors has long been in question. Anatomically they are usually benign, but a few scattered ones have been reported which were histologically suggestive of malignant growth. Likewise in some instances metastases have been recorded; a few resulted in death by local extension, and evidence suggesting metaplasia to carcinoma and sarcoma has been seen in others. McFarland and Patterson considered metastasis to have been proved in only 2 cases (Simmons' cases 2 and 4), and they stated that the growth is "locally malignant but generally benign." On the other hand, McGregor (1934) listed 28 cases as instances of malignant ameloblastoma.

There are 17 cases among those reviewed in which there seem to have been present some evidences of malignancy. However, metastases have been reported, even among the cases in the following list, without histologic examination of the metastatic growth. Some of the reported metastases had not been submitted to even gross pathologic examination. In the following 9 cases metastases appear to have been observed in the presence of ameloblastomas, and the secondary growth probably was ameloblastomatous in nature:

1. Eve: An ameloblastoma of the left mandible with evidence of lumbar metastases (autopsy).

2. Ewing (1922 [*b*]): "In one very extensive fibro-epithelial tumor I found metastases in one cervical node and a small nodule in the lung."

3. Ewing (1922 [*b*]): "In another very malignant case the fourth recurrence was in the cervical nodes and loose tissues of the neck and probably in the lungs."

4. Weissenfels: A histologically confirmed ameloblastoma of the left mandible recurring in the jaw with clinical and roentgen evidence of metastasis in the lung (no autopsy).

5. Simmons (case 2): A gland 4 cm. in diameter removed from the bifurcation of the carotid artery, showing typical ameloblastomatous structure.

6. Simmons (case 7). An ameloblastoma of the right mandible of twelve years' duration accompanied by an ameloblastomatous-like structure in a removed cervical gland and by evidence at death of metastasis in a lung.

7. New (1929): A metastasis from an ameloblastoma of the mandible in submaxillary glands, with no recurrence after removal of the glands and of the primary tumor.

8. Gentsch: An ameloblastoma developing in the left maxilla eleven years after an injury caused by shrapnel and invading the lymph nodes, sphenoid sinus and malar bones.

9. Spring: A metastasis, after many recurrences of a mandibular ameloblastoma, to the frontal bone.

Seven tumors showed histologic pictures of a malignant type, according to the writers who reported them. From the descriptions given they have been classified as carcinoma ameloblasticum and sarcoma ameloblasticum.

10. Heath (case 2234, reported by Eve): Sarcoma ameloblasticum.

11. Krompecher: Sarcoma ameloblasticum.

12. Papadmitriou: Sarcoma ameloblasticum of short duration, recurring three years after partial resection (recurrence reported by Hallner).

13. Allgayer: Carcinoma ameloblasticum.

14. Murphy: Carcinoma ameloblasticum of the left maxilla, appearing five years after injury.

15. Jager: Carcinoma ameloblasticum.

16. Horsley: A growth of the right mandible variously diagnosed as ameloblastoma with malignancy (Bloodgood), epithelioma of the ameloblastomatous type (Broders), and not ameloblastoma (Fox and Stengel).

Another author reports a possibly malignant ameloblastoma:

17. Kotanyi: One (3.84 per cent) of 26 cases reported as instances of malignant growth.

Metaplasia to more malignant growths has been seen by:

1. Heath (1880): An ameloblastoma of the left mandible of thirty-five years' duration with an orange-sized metastasis of sarcomatous type beneath the right deltoid muscle and smaller metastasis in the pelvis. The patient died with round cell sarcoma.

2. L'Esperance (case 8): Metaplasia from ameloblastoma through large alveolar and spindle cell sarcoma and perithelioma to diffuse round cell sarcoma.

3. Bercher and Grandclaude: An ameloblastoma recurring after seven years with metaplasia to basal cell carcinoma.

4. Suker: A squamous cell carcinoma of the orbit considered by the author as originating from an ameloblastoma.

Four other cases have been listed by a number of writers as instances of malignant growth. In a case reported by Kaufmann (1920) a cylindric cell carcinoma appeared near benign cystic remains of an old ameloblastoma, but this may have represented an independent growth. Death resulted from local extension in Simmons' case 1 and in Kegel's case 38746 and case 14752.

Vorzimer and Perla reported a case of ameloblastoma of the lung, confirmed at autopsy, which they considered as probably the result of aspiration of material from an oral ameloblastoma.

Since it is not felt that metaplastic growths, local extensions or implantations should be considered as evidences of malignant growth, only the aforementioned 9 cases of metastasis, 7 cases of histologically malignant ameloblastoma, and Kotanyi's case can be accepted as instances of possibly malignant growth. The 17 tumors listed here represent only 4.5 per cent of the 379 ameloblastomas surveyed. This figure is of the same order as Kotanyi's, which is based on a much smaller number of the tumors (3.84 per cent).

In view of the foregoing findings it appears that ameloblastomas are benign and that the relatively small number of malignant ones are atypical. The ameloblastomas should be treated as expansive benign growths with a tendency to recur. This is borne out by the 119 cases in which from 1 to 22 recurrences were reported. In many cases the recurrence can be traced to incomplete enucleation of the tumor from the numerous crypts and lacunae within the bone. These findings agree with Ewing's statement (1922 [*b*]) that "in spite of their relatively benign structure, the prognosis in this group is unfavorable." They are persistent, not malignant, tumors.

ETIOLOGY

From the earliest writings it has been reported that the ameloblastoma is in some way derived from some part of the odontogenic apparatus or from cells with a potentiality for forming the odontogenic tissues. Broca advanced the theory that "the new group of tumors designated by the name of odontomes might arise from an overgrowth of the dental germ." Falkson, who observed the similarity between the enamel organ and this tumor, presented the theory that a surplus of tooth buds might be formed along with the normal buds and might give rise to the neoplasm.

Buchtemann (1881) announced his belief that these tumors arose from the oral mucous membranes or from the mucous glands. Eve claimed that they resulted from an ingrowth of the gum epithelium.

Malassez observed circular or oval cordlike and glandlike groups of epithelial cells about the roots of teeth. These he considered as vestiges of the rich dental apparatus of lower vertebrates, and he gave to them the name *des débris épithéliaux paradentaires.* These cell roots, Malassez claimed, become proliferative and give rise to epithelial growths about the jaws, including ameloblastomas. Since Malassez' original demonstration of the epithelial débris a number of sources have been shown to supply the epithelial cells. It is generally conceded that the sheath of Hertwig,[3] after it has fulfilled its function of outlining the future root,

3. Robinson, H. B. G.: Arch. Path. **23**:664, 1937 (fig. 1 *A*, 3 *h*).

and the remnants of the dental lamina [4] may be the chief sources of the paradental remains. This theory that ameloblastoma has its origin in epithelial débris has been accepted rather widely. Kegel has been mentioned by Geschickter as not accepting the theory that paradental débris is the origin for this growth. It is inferred that this argument is based on the apparent derivation of the cells of the sheath of Hertwig from the outer epithelial layer and not from the inner or ameloblastic layer. However, the cells of the epithelial rests may be derived from the cells of the dental lamina which have not yet differentiated to a point where their potentiality is restricted to the cell type of the inner or the outer epithelial layer. Moreover, the sheath of Hertwig, according to Orban and others, is formed by the union of the internal and external epithelial layers of the enamel organ.

Bland-Sutton did not agree with any theories attributing this tumor to the development of embryonal cells, because he considered it a tumor of middle life.

Cahn observed an ameloblastic structure on the wall of a dentigerous cyst, a finding which he considered as indicative of origin of the ameloblastomas from dentigerous cysts. Attention had been drawn to this possibility by Barrie, who published a report on a dentigerous cyst lined with adamantine epithelium; by Hertzler, who pointed out that odontoid cysts seemed to be "a connecting link between the adamantinomas and the cysts"; by Morlet and Morlet, who described a tumor intermediate between these two types, and by Schroff, who described a similar one. Churchill (1934) claimed that, although the walls of dentigerous cysts may become erratic in behavior and show proliferations comparable to the ameloblastic layer and suggestive of ameloblastoma, these two dentocystic growths are separate entities and histologically distinct. He emphasized as a distinguishing point that vascularization had not been encountered in the follicles of true ameloblastomas. Jay and Hofrath also felt that these two lesions could be histologically differentiated.

Broders and MacCarty considered the ameloblastoma as closely resembling the squamous cell carcinoma. While I have not seen any growths showing such characteristics, I have noted a similarity to the basal cell carcinoma in one section.[5] A number of authors have pointed out this similarity of structure (Sprawson and Keizer; Tratman). Tratman, commenting on the type of epithelium in the ameloblastomas, stated:

> All types of epithelium occur from the squamous type up to the specialized adult columnar ameloblastic type of cells arranged as linings to cystic cavities with other adjacent cells resembling the stratum intermedium and stellate reticulum of the normal enamel organ. However, these ameloblast-like cells have no Tomes' processes and the location of their nucleus is different. They are in fact practically

4. Robinson [3] (fig. 1 *A 2f*).

5. Robinson [3] (fig. 2 *A*).

ordinary columnar epithelium lining a cystic cavity and accidentally associated with cells resembling the stratum intermedium and stellate reticulum of the normal enamel organ.

A study of a section of solid ameloblastoma which I [6] have and of a similar section which Kronfeld had hardly justifies the conclusion that the association of these cells is accidental. The nuclei in the ameloblast-like cells are in a migratory position as in the normal enamel organ just before it assumes its function. Tomes' processes do not ordinarily appear before the enamel is being formed, and they cannot be expected in the nonfunctional neoplasm. In my section the margin of the ameloblast-like cells is scalloped externally, suggesting the future scalloped dento-enamel junction that would appear if the cells laid down enamel. The two growths usually differ in biologic activity. The ameloblastoma usually degenerates, with cystic centers to its follicles, grows deep within the bone, expanding a thin shell about its mass, and is commonly seen in the lower jaw but is rare or unknown on the face, neck and scalp. On the other hand, the basal cell carcinoma tends to ulcerate, remains superficial and is rare or unknown in the mandible and commonly seen about the face, neck and scalp. On the basis of these morphologic and biologic characteristics I feel justified in classifying these two neoplasms as distinct entities.

Irritation has been considered as a factor in the genesis of the ameloblastoma. Wohl cited Partch as believing that this neoplasm is of inflammatory nature and further elucidated this aspect. New (1915) correlated the predilection of this growth for the posterior part of the mandible with the difficulty of eruption of the third mandibular molar. He further cited the average age in cases coming under his observation, i. e., 33½ years for those appearing in the molar region and 16½ years for those appearing in the canine area. These ages are compared with those for the normal eruption of the third molar and the canine tooth, 16 years and up and from 10 to 12 years, respectively. He interpreted these data as suggesting that "the eruption of the teeth may play an important part in the chronic irritation which is probably necessary to stimulate the growth of these tumors." Ghosh also considered this factor, finding a great number of ameloblastomas associated with unerupted teeth and accounting for the comparative rarity of this growth in the maxilla on the basis that this bone has better drainage and is less subject to dental sepsis as compared with the mandible.

Geschickter assumed a probable relationship between rickets and ameloblastomas. Colored patients, he observed, commonly demonstrated both of these disturbances, and the dietary deficiency in rickets might account for the tumor through disturbance of the enamel organ. Irregularity of the ameloblastic layer and budding off of cells were noted

6. Robinson [3] (fig. 1 *C*).

in animals kept on a rachitic diet for nine months. No ameloblastomas were produced experimentally.

The essentials of these theories may be tabulated as follows:

1. The ameloblastoma takes origin from the odontogenic apparatus:
   (*a*) From overgrowth of the buds or from excess of buds
   (*b*) From the oral epithelium
   (*c*) From epithelial débris
   (*d*) Indirectly through dentigerous cysts
2. Stimulation for the growth may result from:
   (*a*) Irritation
   (*b*) Dietary disturbance.
3. The ameloblastoma is a form of:
   (*a*) Squamous cell carcinoma
   (*b*) Basal cell carcinoma.

It seems reasonably certain that the primordium for this neoplasm is some part of the odontogenic apparatus or cells with a tendency to form this apparatus. This conclusion is based chiefly on the morphologic characteristics. The periods of formation of the anlagen of normal tissue and of associated embryonic rests have been given by Li and Yang. They place the period of origin of the enamel organ and of the ameloblastoma in the sixth week of embryonic life. Whether this primordium is actually an excess of tooth buds, an overgrowth of an individual bud, oral epithelium or epithelial débris in any of its forms has not been demonstrated. The argument advanced by Bland-Sutton that this growth cannot arise from embryonal cells is refutable on two counts: 1. Tumors arising from embryonal cells do occur at late ages; for example, hypernephromas, adenomas and mixed tumors of the salivary glands. 2. The ameloblastoma is not a growth restricted to middle life, as shown by my studies of the incidence according to age.

Churchill's thesis more or less rules out any transition from dentigerous cysts to ameloblastomas. Geschickter's theory seems to present a rather wide generalization based on observation of disturbed enamel organs in animals made rachitic experimentally and on the incidence of rickets and tumors in Negroes.

The theory that irritation has incited the primordium to proliferate does not appear to be ungrounded. A summary of the cases recorded in the literature shows:

44 cases preceded by extraction of teeth
9 cases preceded by injury to the teeth or jaws
21 cases preceded by oral infection
14 cases with unerupted teeth contained in the mass
4 cases associated with impacted teeth
1 case associated with tooth supposed to have been extracted
1 case associated with a supernumerary tooth on the opposite side
1 case in the position of an unerupted tooth
5 cases preceded by dentigerous or dental root cysts
1 case in a patient who kept snuff in a gingival fold
1 case ascribed to "treatment of patient's teeth"

As many of the histories in these cases suggest a traumatic or irritating factor in the form of surgical disturbance, injury, infection, impaction or difficulty of eruption, as well as an oral disturbance in some instances, it seems that there may be a primordium of cells with a tendency to form the epithelial portion of the tooth germ, which may be excited to growth by some irritating secondary factor.

COMMENT

Churchill's classification (1932) of the ameloblastoma as a dentocystic tumor derived from the odontogenic apparatus of the squamocellular mucosa seems to be the most adequate of those encountered. Likewise his subdivision into benign ameloblastoma without or with cornification in solid, cystic or melanotic form and the maligant sarcoma ameloblasticum and carcinoma ameloblasticum covers most of the types that have been described. The term "adamantinoma" is suggestive of a hard tumor of enamel, and the descriptive name "ameloblastoma," proposed by Ivy and Churchill (1929), is preferable for this epithelial growth.

A review of the literature shows that the ameloblastoma is not just a pathologic or clinical curiosity but is of more common occurrence than most textbooks lead one to believe. No sexual preference can be assumed on the basis of the 311 reported cases, 54.3 per cent of which ocurred in females. The descriptions in the literature do not allow any conclusions to be drawn in regard to racial predilection. The studies on incidence according to age are more fruitful. The average age at the time of discovery, which approximates the age at the time of onset of the growth and which actually represents the age at which symptoms appear, is 30.1 years (222 cases). The fact that 70 per cent of these tumors first made their appearance between the tenth and the thirty-fifth year indicates that the common conception of this growth as one of middle life is not exactly correct. This error probably arose through basing computations on the age at the time of reporting, given by the reporting authors, which for 248 cases averages 37.6 years. The duration may be surprisingly long—in one instance, fifty-three years (Zatti, 1894)—and averages 8.5 years (232 cases). The mandible is the more common situation for the ameloblastoma, 83.7 per cent of 295 cases having been reported to have occurred in this situation. Most of the growths were cystic at the time of operation; some were cystic and solid, and the smallest number were solid.

Ten of the reported metastases seemed to have some basis for the diagnosis. Seven of the ameloblastomas reported as histologically malignant can be included as possibly malignant. Seventeen cases, therefore, may be considered as instances of malignant ameloblastoma. These

represent only 4.5 per cent of the total number of instances of ameloblastoma collected, and the percentage compares favorably with Kotanyi's figures. McGregor (1935) considered many more cases as instances of malignant ameloblastoma, but I cannot concur in her conclusions. Many of these often cited cases represent instances of metaplasia to malignant tumors of epithelial or connective tissue type, local extensions resulting in death or independent tumors. While ameloblastomas are not typically malignant, their prognosis is unfavorable because of the difficulty of complete removal and their persistent nature.

Etiologic considerations are at best based only on theoretical grounds in view of the present inadequate knowledge of the dentocystic tumors. It does seem that the ameloblastomas arise from some part of the odontogenic apparatus or cells with a potentiality for development into tooth-forming tissues. This primordium may be incited to proliferate by irritation from retained teeth, surgical operations on the teeth or jaws, injury, infection or possibly general disturbances of the economy.

SUMMARY

The ameloblastoma never forms enamel and is not an extremely hard tumor. The term "adamantinoma" is therefore a misnomer. At least 379 cases of ameloblastoma have been reported; 54.3 per cent of 311 ameloblastomas occurred in females. Inadequate data do not permit determination of any racial preference. The average age at the time of reporting, a nonsignificant figure, is 37.6 years (248 cases).

The average duration (the difference between the age at the time of reporting and the age at the time of discovery) is 8.5 years (232 cases). The average age at the time of discovery, a clinically significant figure, is 30.1 years (222 cases); in 70 per cent of these cases the growth was first noticed between the tenth and thirty-fifth years.

Of 295 ameloblastomas 83.7 per cent occurred in the mandible. Only 4.5 per cent of the 379 tumors reviewed showed metastases or histologic evidence of malignancy.

While the ameloblastoma is a benign neoplasm, it is extremely persistent and tends to recur after operative intervention. Recurrence was noted in 119 instances.

Irritation in the form of retained teeth, infection, injury, operative trauma or general disturbance seems to be an etiologic factor, stimulating a primordium with a potentiality for the formation of the enamel organ.

Case Reports

HODGKIN'S DISEASE OF THE STOMACH WITH FATAL GASTRIC HEMORRHAGE

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Alterations in the gastric wall in Hodgkin's disease are rare. In 1911 Fabian [1] in a review of lymphogranulomatosis (Hodgkin's disease) called attention to the fact that not a single acceptable instance of involvement of the stomach had been reported to that date. The first definite case was presented by Schlagenhaufer, briefly in 1913 and in detail in 1920.[2] Reports of primary or predominant gastric involvement have since appeared with increasing frequency, so that it has been possible to gather twenty-two instances confirmed by necropsy from the literature.[3] In some instances the histologic descriptions are lacking in detail or are not included [4]; in one, access to the original publication is not available.[3k].

The gross appearance of the stomach was not uniform. Diffuse infiltrations, bulky masses and ulcerations were observed singly or in combination. In five cases [5] necropsy demonstrated that the stomach was the only organ involved.

The case now reported in which Hodgkin's disease predominantly involved the stomach is of unusual interest because of an asymptomatic course which was abruptly terminated by fatal gastric hemorrhage.

On March 5, 1936, a 54 year old white man was admitted to the Third (New York University) Surgical Division of Bellevue Hospital for removal of a

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4. Henke and Lubarsch.[3l] Tschilow.[3m]

5. Mittelbach.[3a] Barsony and Koppenstein.[3b] Dudits.[3c] Baumgartner.[3d] Singer.[3n]

painless, progressively enlarging mass of the anterior thoracic wall. He denied having any other complaints, although later it was possible to elicit a history of occasional gaseous eructations during the preceding eight months.

He appeared well developed and well nourished. Over the left third intercostal space, at the left sternal border, a mass, 4 by 6 cm. in diameters, was palpated. This was firmly attached to the underlying structures but was covered by normal, freely movable skin. The edge of the liver was palpated 4 fingerbreadths below the xiphoid cartilage, and the spleen was felt 3 fingerbreadths below the left costal margin. The superficial lymph nodes were not enlarged.

The urine was normal. The red blood cell count was 4,800,000, and the hemoglobin was 80 per cent. There were 11,500 white blood cells per cubic millimeter, with a normal differential count. The Wassermann reaction of the blood was negative.

Four days after admission, the patient suddenly vomited about 300 cc. of bright red blood. Despite the usual regimen for hematemesis and supportive measures, including two transfusions, he continued to vomit large amounts of blood and died on March 11, two days after the onset of bleeding. The temperature was normal until just before death, when it rose to 104 F.

At autopsy the mass in the wall of the chest was closely adherent to and invaded the lateral border of the sternum and was intimately attached to the left third and fourth costal cartilages on their anterior and posterior aspects, although it was easily separated from the overlying fascia and muscle. No connection between the mass and the mediastinal contents could be demonstrated.

The stomach was dilated and contained a liter of clotted blood. A circular ulcer, measuring 7 cm. in diameter, was present on the lesser curvature, extending from a point about 1 cm. below the cardia and involving both the anterior and the posterior wall (fig. 1). The margin was thickened and elevated. The base consisted of firm, grayish white, tumor-like tissue, 1 cm. in thickness, covered externally by smooth, shiny serosa and on the mucosal aspect by blood-tinged, necrotic débris. No point of former bleeding was found. On section, the firm white tissue extended beyond the margin of the ulcer to merge gradually into normal gastric wall; the tissue was covered by smooth mucosa studded with tiny superficial ulcerations. A similar ulcer, measuring 2 cm. in diameter, was found immediately below the one described. The remainder of the stomach appeared to be normal save for the absence of rugae.

The esophagus and the small and large bowel, as well as the mesenteric nodes, were not involved. The peripancreatic nodes were enlarged, varying from 0.5 to 5 cm. in diameter; they were fleshy in consistency, a glossy light brown in color and discrete, with the exception of a few fused packets closely adherent to the body of the pancreas and to the splenic artery and vein at the hilus of the spleen.

The spleen measured 20 by 10 by 6 cm.; it was firm and studded with circumscribed white nodules averaging 2 cm. in diameter and containing scattered hemorrhagic foci.

The portal nodes, periaortic nodes and the nodes along the lesser curvature of the stomach were the only other ones affected, and they were small and discrete. None showed caseation or calcification.

Microscopically the floor of the ulcer in the stomach was composed of a loose vascular connective tissue stroma with a diffuse polymorphous cellular deposit (fig. 2). In most sections lymphocytes predominated; in slightly lesser number were found mononucleated giant cells from 20 to 40 microns in diameter, with relatively little basophilic cytoplasm and large round and irregular vesicular

nuclei, many of which contained single central nucleoli. Giant cells with from 2 to 8 nuclei were common. Fibroblasts were numerous while plasma cells and eosinophils appeared less frequently. Toward the edge of the infiltration, the thickness of the gastric wall diminished, and individual layers could be identified. Here dense collections of lymphocytes, many eosinophils and few giant cells were found in the submucosa and subserosa, while similar cellular infiltrations separated individual muscle bundles of the muscularis.

Fig. 1.—Stomach with a large crater-like ulcer and a single smaller ulcer on the lesser curvature.

All the altered abdominal nodes showed loss of the normal architecture, but the resultant picture varied considerably even in a single node. Foci of densely packed lymphocytes merged with areas of fibrosis and hyalinization in which mononucleated and multinucleated giant cells were numerous. Fibroblasts, too, were common, but plasma cells were scarce, and eosinophils were not encountered. The picture in the nodes at the hilus of the spleen was closest to that in the stomach. Invasion of the capsule and body of the pancreas was observed in a few instances.

The splenic nodules were separated from the splenic substance by a poorly defined hemorrhagic zone and were composed of dense connective tissue stroma with a few recognizable malpighian corpuscles and foci of hemorrhage and necrosis. Lymphocytes, fibroblasts and mononucleated and multinucleated giant cells were found in great numbers.

The microscopic structure of the tumor-like nodule in the wall of the chest was similar to that of the lymph nodes and the wall of the gastric lesion. The marrow of the sternum in the region of the nodule just referred to was for the most part replaced by fibrous tissue but in a few locations showed the same microscopic picture as the nodule.

Fig. 2.—Photomicrograph of the base of the ulcer in the stomach, illustrating the pleomorphic character of the cells.

Sections stained with the Ziehl-Neelsen technic did not reveal any acid-fast organisms.

COMMENT

Although occult blood has frequently been found in the stool in Hodgkin's disease of the stomach, hematemesis has occurred in only five instances,[6] and death due to massive gastric hemorrhage, in only a single case.[2b]

6. Schlagenhaufer,[2b] Mittelbach,[3a] Coronini,[3e] Sussig,[3f] Hayden and Apfelbach.[3g]

The absence of prominent gastric symptoms in the case reported here is noteworthy. In most instances the gastric lesion was accompanied by localizing symptoms, of which pain, nausea and vomiting were the most distressing. This case is also an exception in the absence of constitutional symptoms and of enlargement of the superficial lymph nodes, although Symmers [7] has shown that enlargement of the thoracic or abdominal lymph nodes may reach remarkable proportions long before the superficial lymph nodes become visible or palpable and that Hodgkin's disease may lead to death without involvement of the superficial nodes.

SUMMARY

A case of Hodgkin's disease is presented which involved the stomach, peripancreatic lymph nodes, spleen and anterior wall of the chest. Constitutional symptoms were absent, and the lesion in the stomach gave little indication of its presence until massive gastric hemorrhage resulted in death.

7. Symmers, D.: Am. J. M. Sc. **167**:313, 1924.

Laboratory Methods and Technical Notes

A SIMPLE MAILING CONTAINER FOR SLIDES

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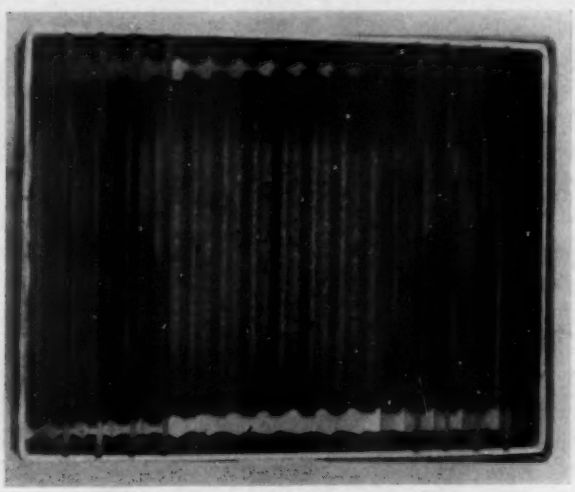
Passed Assistant Surgeon, U. S. Marine Hospital

Mailing microscopic slides from one institution to another involves risk of breakage, expense of a mailing container and danger of freshly prepared slides being spoiled by sticking together. My co-workers and I have not seen our method described elsewhere or in general use. We claim no originality for its use but feel that a description of it may help some one who is faced with the problem of finding a satisfactory container for mailing slides before the cover glass cement is dry.



A mailing container for slides.

New microscopic slides as furnished by the manufacturer usually come in a cardboard box 4 by 3 by 1 inch (10 by 7.5 by 2.5 cm.). When the box is empty we save it and cut a piece of corrugated paper to fit each long side and the bottom of the box as shown in the photograph. The completed stained and mounted tissue slides are placed in the slots formed by the corrugated paper, and the cover is placed over the box. The slides fit tightly and cannot move out of place.

The box has the additional advantage of being usable in the small laboratory for a file box, saving the expense of wooden boxes, which cost from 15 to 75 cents each. Twenty slides can be mailed in one box. We have used this type of container for two years with no appreciable breakage.

General Review

TECHNICAL METHODS FOR THE STUDY OF BLOOD PLATELETS

A CRITICAL REVIEW WITH BIBLIOGRAPHY

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There are technical methods for studying the platelets (1) inside and (2) outside the body.

In man, the platelets have not been studied in vivo; as yet this method is confined to animals. In mammalians, the wing of the bat, the mesentery and omentum of the guinea-pig, rat and rabbit, and the pia vessels of the cat and guinea-pig have been used. For detailed directions consult: Bizzozero (1882 [*a* and *b*]); Eberth and Schimmelbush; Laker (1889); Hayem (1923); Jolly, and Forbes. The technic of placing a window in the ear of a rabbit, described by Sandison and by Clark, renders possible observations for a prolonged period of time, while other technics make it possible to observe the circulation in ordinarily inacessible organs, such as the spleen (Knisely) and the lung (Wearn and others).

In reptiles, one may use the mesentery, lung, tongue and urinary bladder. The mesentery of the curarized frog is especially suitable for such observations (Hayem, 1923). It is generally believed that platelets do not exist in their usual numbers in the lower forms (birds, reptiles) and that the cells that resemble them in *physiologic* behavior are the thrombocytes.

ENUMERATION OF PLATELETS OUTSIDE THE BODY

One hundred and sixteen different methods of counting platelets were reviewed. In general, these methods may be divided into two main classes: (1) those using venous blood; (2) those using blood from a cutaneous puncture. Each one of these classes of methods may be subdivided into indirect and direct methods.

In the indirect methods the ratio of platelets to red blood cells is determined in a given sample of blood; then the number of red blood cells per cubic millimeter of blood is determined in another sample. The

Aided by a grant from the J. Ewing Mears Research Fund.

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manner of establishing the ratio between the platelets and red blood cells varies with different authors. Some dilute the outcoming blood and place a very small amount of the diluted blood between a slide and a cover slip; then they count the number of red blood cells and the number of platelets in succeeding fields until from 250 to 1,500 erythrocytes have been counted. Others use a ruled counting chamber, count (1) the platelets and (2) the red blood cells in the ruled areas alone and calculate the ratio between the respective totals. Others establish the ratio on stained fixed dry smears (Fonio, 1912; Yamamoto).

In the direct methods the platelets are counted directly in a manner similar to that used in counting erythrocytes.

Only a more detailed account could give an accurate idea of the number of methods and their modifications introduced into the technic of platelet counting. For further details consult: Kemp, Calhoun and Harris; Fonio (1912); Kristenson (1924); Baunack, and Olef. The following are representative examples of the various methods:

Counting of Platelets in Venous Blood.—1. Teoumine Direct Method: Using a finely graduated 0.5 cc. tuberculin syringe, dilute the blood 1:1 with a sterile 5 per cent sodium citrate solution. Use a fine needle and push the sterile solution into the very end of it before inserting it into the vein. Empty the diluted blood into a small vessel and with an erythrocyte pipet make the platelet count directly from a 1:100 dilution of the mixture, using the 5 per cent sodium citrate solution as the diluent and the standard counting chamber.

2. Thomsen Direct Method (Modified by Reimann): Draw 0.9 cc. of blood from the vein with a no. 20 needle into a tuberculin syringe containing 0.1 cc. of a 10 per cent sodium citrate solution. Remove the needle from the syringe and invert the latter several times to mix the blood with the solution. Close the open end of the syringe with a piece of rubber tubing fastened to the barrel by rubber bands; remove the plunger and place the syringe in a vertical position to permit sedimentation. After this has occurred, make a 1:20 dilution of the supernatant fluid with physiologic solution of sodium chloride. Place a drop of this dilution in a counting chamber and count all the platelets in 80 small squares. Multiply the total number counted by 1,000. The result is the number of platelets per cubic millimeter of plasma. Centrifugate the syringes containing the blood for twenty minutes at 2,000 revolutions per minute. Record the relative amount of plasma. Calculate the number of platelets per cubic millimeter of blood thus:

$$X = \frac{\text{number of platelets}}{\text{per cubic millimeter of plasma}} \times \frac{\text{amount of plasma}}{\text{total amount of mixture}}$$

To the result, 10 per cent of the total number should be added to offset the original dilution with citrate solution.

See also modifications by Gram, Petri, Windfeld and Nygaard and adaptations to cutaneous blood by Spitz, Thomsen (1923) and Jurgens (1934). Other useful direct methods have been contributed by Buckman and Hallisey, Kristenson (1922) and Gutstein.

3. Aynaud (1910 [*c*]) Indirect Method: Place about 0.5 cc. of a 10 per cent sodium citrate solution in a syringe and aspirate from 1 to 2 cc. of blood into

the solution. Mix well and place in a paraffin-coated tube. Make an erythrocyte count on the blood dropping from the needle, before removing it. Dilute the blood further with the following solution: sodium citrate, 2 Gm.; sodium chloride, 1 Gm.; distilled water, 100 cc., and a 40 per cent solution of formaldehyde, 2 cc. Establish the ratio of platelets to erythrocytes in a counting chamber after counting between 1,200 and 1,500 erythrocytes. Calculate the number of platelets per cubic millimeter of blood from the erythrocyte count and the erythrocyte-platelet ratio.

4. Preiss Indirect Method (Slightly Modified): A diluent is used, made as follows: Tyrode solution is prepared without dextrose, filtered and autoclaved; to 4.5 cc. of this is added 0.5 cc. of a clear centrifugated sterile solution of heparin (heparin, 0.1 per cent, in 0.9 per cent sodium chloride solution). Place 0.5 cc. of this diluent in the syringe, up to the very end of the needle; aspirate a little blood and mix with the remaining 4.5 cc. of the solution in a paraffin-coated vessel; with a paraffin-coated rod place a drop of the mixture in each of the two sides of a Burker counting chamber. Find the ratio of erythrocytes to platelets in 25 squares on each side of the chamber. From this ratio and the erythrocyte count per cubic millimeter of blood the total platelet count is calculated.

5. Cramer-Bannerman Indirect Method: This is another useful indirect method for use with venous blood.

Counting of Platelets in Cutaneous Blood.—1. Rees-Ecker Direct Method: Aspirate the following freshly filtered solution up to point 0.5 of a pipet for counting erythrocytes: sodium citrate, 3.8 Gm.; 40 per cent formaldehyde solution, 0.2 cc.; brilliant cresyl blue, 0.1 Gm., and distilled water, 100 cc. Make a puncture from 3 to 4 mm. deep in the finger and from the freely bleeding wound aspirate enough blood to push the column up to point 1. Fill the remainder of the pipet with the solution up to point 101. Shake, place a drop in the counting chamber, wait from fifteen to twenty minutes for settling and then count the platelets in the entire finely ruled area (400 squares). Multiply the total by 2,000 to obtain the number of platelets per cubic millimeter of blood.

Other useful direct methods are those of Hayem (1878); Afanassiew; Wright and Kinnicutt; Ottenberg and Rosenthal; van Herwerden; Thomsen (1923); Leake and Guy; Van Goidsenhoven; Schenk; Kristenson (1928); Goadby, and Jurgen's modification of the Thomsen method.

2. Author's Indirect Method: Place a large drop of freshly filtered Rees-Ecker solution over the skin of the finger. Puncture the skin through the liquid, remove a small amount of the blood-liquid mixture, place it on a cover slip, invert this over a slide and ring the edges with petrolatum. With an eyepiece equipped with cross hairs, count the platelets and erythrocytes in successive fields until at least 1,000 erythrocytes have been counted. Use a 10 × ocular and a 40 × or 90 × objective, preferably dry, to avoid pressure on the preparation. Establish the ratio of platelets to erythrocytes. From this ratio and the simultaneously performed erythrocyte count, the absolute number of platelets per cubic millimeter of blood is calculated. The Cramer-Bannerman solution or the citrate-formaldehyde solution suggested on page 859 may be used as the diluent instead of the Rees-Ecker solution.

Other indirect methods are those of Fusari; Determann; Pratt (1905); Sahli; Port and Akiyama; Degkwitz; Pagniez and Mouzon; Bannerman; Mackay, and Olef.

3. Fonio (1912) Indirect Method on Dry Smears: Place a large drop of 14 per cent magnesium sulfate solution over the skin of the finger, which must be clean and dry. Puncture the skin through the liquid, collect a drop of the blood-liquid mixture with a paraffin-coated glass rod, place it on a clean slide and make a smear. Fix the smear in methyl alcohol (three minutes) and stain for from three to four hours with a diluted Giemsa solution (15 drops of stock Giemsa solution added to 10 cc. of distilled water). Wash with distilled water, dry and examine with the oil immersion lens. Establish the ratio of platelets to erythrocytes from at least 1,000 erythrocytes. Calculate the number of platelets from this ratio and an erythrocyte count done at the same time.

Other similar methods are those of Osseladore (1925); Gaspar; Hittmair, and Steinmaurer.

The available methods are described in the order of their preference for persons experienced in laboratory work. The choice of methods obviously must depend to a certain extent on the experience of the one who is to use the method. For inexperienced workers, the order of choice should be: (1) indirect platelet count on cutaneous blood with an isotonic solution containing a stain, a fixative and an anticoagulant; (2) indirect count on venous blood; (3) direct platelet count on cutaneous blood; (4) direct platelet count on venous blood. The ratio of erythrocytes to platelets should be established from at least 1,000 erythrocytes. The choice of methods is further conditioned somewhat by the type of blood to be examined. With leukemic and thrombopenic blood, counting on wet specimens should be supplemented by a method in which the dry stained smear is used, such as Fonio's (1912), as a further check on the number of platelets or as a means of avoiding the inclusion of any hyaline, platelet-like bodies in the total count. Venous blood is preferable to cutaneous blood. The trauma involved in an incision of the skin is greater than that in a venous puncture with a small needle (from 26 to 27 gage). When one considers the rôle played by the platelets in repairing the effects of trauma, it is logical to anticipate that the trauma involved in an incision of the skin will affect the number of the platelets. Comparative determinations of the platelets in vessels with and without trauma yield great differences (Lucia and Rickard; unpublished observation by Tocantins), and this fact renders serial counts from the same location within short periods of time of questionable value. Appreciable numbers of platelets are lost in the lips of the wound before the blood reaches the surface. With a technic such as the Teocumine or the Preiss, the blood passes from the lumen of the vessel directly into the lumen of the needle, where it is immediately diluted and mixed with the anticoagulant and (in a technic to be described) fixed, all surfaces of contact having previously been wetted by the solution. Counts done on venous blood are more representative of the actual conditions in the circulation, for a larger amount of blood

is usually collected for examination than when a cutaneous puncture is used. It is also possible to repeat the count and make observations on the other morphologic constituents of the blood.

For greater accuracy, in experienced hands, direct methods are preferable to indirect ones. Most of the objections usually raised against the use of the direct method apply only if it is employed by one who is inexperienced with the manipulative procedures involved. Even on cutaneous blood, if there are free bleeding from the wound, rapid coordination of movement, use of scrupulously clean glassware and solutions, duplicate determinations and uniform shaking, this method is useful.

Some of the objections to the indirect method of counting, which was first proposed by Laker (1886) and Kemp (1886), are: The mixture of blood and diluting solution is seldom, if ever, uniform and not the same each time. The ratio of erythrocytes to platelets in a given sample of blood determined on the counting chamber often varies widely from that determined in wet smears (Lucia and Rickard). Platelets and erythrocytes are not distributed evenly through the preparation since no provision is made for shaking before counting. It is obvious that the greater the number of erythrocytes and platelets counted, the more accurate will be the ratio established between these two elements; if a counting chamber is used to establish this ratio, the total number of erythrocytes counted will vary, depending on the dilution employed. The method has defects intrinsic in any determination done indirectly, that is, in relation to another equally changeable element. The greatest source of error, however, is in the assumption that platelets and erythrocytes keep an even proportion in numbers toward each other between the two main steps of the method. The markedly different physical properties (adhesiveness, specific gravity, size and others) of platelets and erythrocytes lead to continuous changes in this ratio. Finally, the proportion of platelets to erythrocytes varies at the same time in different portions of the circulation (Tocantins, 1936 [*f*]), and this variation is even more marked within short spaces of time in capillaries and venules (Beecher).

With the Thomsen (1920) method and its modifications, the platelets are counted in the plasma, free from other cells. This is advantageous in some respects; it decreases the possibility of confusion with the products of the disintegration of erythrocytes besides having all the other advantages of methods in which venous blood is utilized. Although the Thomsen method at first seems a direct method of counting platelets in whole blood, it actually is an indirect method, for one must always have a supplementary determination of the volume of packed cells or of the hemoglobin (Gram) to calculate the absolute number of the platelets per cubic millimeter of blood. This multiplicity of determinations may contribute to increase the error, but, unlike the indirect method

in which use is made of the erythrocyte-platelet ratio, the determinations are all done in the same sample of blood. There is also some question as to the stability and uniformity of the suspension of platelets in plasma during the time required for sedimentation of the erythrocytes (Baunack; Olef).

As anticoagulants, sodium citrate, sodium oxalate (Brodie and Russell; Leake and Guy), sodium metaphosphate (Pratt, 1903; Aynaud, 1910 [*b*]; Helber; Olef), sodium (Hayem, 1878), magnesium sulfate (Fonio, 1912; Gutstein), heparin (Casey; Preiss), peptone (Afanassiew) and gelatin (Port and Akiyama) have been used in diluting solutions. Of these, sodium citrate seems to be the best. Oxalates often form crystals and precipitates with the plasma. Sodium metaphosphate should be in higher concentrations (from 5 to 10 per cent) than those generally used (2 per cent). Heparin solutions are difficult to make clear even after prolonged centrifugation. Peptone and gelatin, like sucrose and dextrose, encourage the growth of bacteria. None of these anticoagulants prevents morphologic changes in the platelets without the addition of a fixing reagent. The preservation of the morphologic character of the platelet for total and differential counting may be obtained only by the dual process of simultaneous prevention of coagulation of the blood and rapid fixation. Good fixation depends not so much on a high concentration of the fixative as on the promptness with which even a weak fixative comes in contact with the platelets after these have left the vessels. As fixing reagents, mercury bichloride (Hayem, 1878; Hofmann), 1 and 2 per cent osmic acid (Fusari; van Emden), solution of formaldehyde U. S. P. (Kemp, Calhoun and Harris), methyl and ethyl alcohol (Brodie and Russell) and potassium bichromate (Determann) have been used. Of these, solution of formaldehyde U. S. P. in a concentration of about 0.1 per cent seems the best. Diluting fluids containing this solution of formaldehyde in percentages of 2 and 2.5 per cent (Kemp and Calhoun; Leake and Guy) often hemolyze erythrocytes and form precipitates with the plasma. It is important to use neutral solution of formaldehyde in the preparation of solutions, for if enough formic acid is present it will hemolyze the red blood cells. When a solution containing formaldehyde has stood for a time, some of the formaldehyde is oxidized and formic acid is formed. When this acid reaches a concentration of from 0.05 to 0.075 per cent it decolorizes the erythrocytes even in isotonic salt solution, although it does not affect platelets or leukocytes (Kemp, Calhoun and Harris). The hemolysis that is occasionally encountered when one is using the Rees-Ecker solution is chiefly due to this. When a diluting fluid containing formaldehyde begins to hemolyze the red blood cells it should be discarded, and the glassware that has been in contact with

it should be thoroughly cleaned. Methyl and ethyl alcohol are poor fixatives for platelets in wet preparations, for they precipitate the plasma proteins and cause secondary changes in the platelets. Mercury bichloride in a concentration such as that in Hayem solution B (0.25 per cent) seems a good fixative. In higher concentration it often produces protein precipitates. Osmic acid, while an excellent fixative for dry smears, is expensive and often produces clumping of erythrocytes in wet preparations. Approximately half of the diluting solutions proposed do not include a fixative.

The inclusion of a stain in the diluting fluid is not necessary except for beginners or in certain pathologic conditions. Brilliant cresyl blue, methyl violet, methyl green, neutral red, methylene blue and eosin have been used. Brilliant cresyl blue in a concentration of from 0.05 to 0.025 Gm. per hundred cubic centimeters seems the best. Methyl violet is satisfactory, while the others, like methyl green, stain the platelets poorly or not at all. For counting on dry smears, the Giemsa or Wright stain demonstrates the platelets best. Stains aid the reading of the meniscus in pipets and syringes but increase the chances of producing artefacts and the labor of cleaning glassware.

The use of diluting solutions that hemolyze the red corpuscles is generally inadvisable. The erythrocyte count which should accompany every platelet count is made impossible, many artefacts are created, and it is difficult to be sure that the platelets themselves are not sometimes affected. In experienced hands, however, these solutions may be used advantageously. The hemolyzing agents employed have been potassium cyanide (Wright and Kinnicutt) and urea (van Herwerden; Kristenson, 1922).

Diluting solutions resembling the plasma in composition, such as Tyrode and Ringer solution, offer no especial advantage in the counting and morphologic study of intact platelets. Their similarity to plasma in composition may be a handicap in this study, for there are few fluids in which platelets disintegrate more rapidly than in plasma collected without precautions to prevent its coagulation. Adding a fixative, such as mercury bichloride, to Tyrode solution (Flossner) remedies the disadvantages of this solution partially, but such an addition obviously alters its delicate chemical balance, thus defeating the original purpose in using it and making its use no more advantageous than that of other far simpler solutions. Both Tyrode and Ringer solution encourage the growth of bacteria; neither keeps well, and the multiplicity of chemical substances in each is a constant source of artefacts. Methods in which Tyrode diluting fluid is utilized invariably yield high platelet counts on cutaneous blood (Flossner; Hofmann; Preiss; Cumings; Horwitz, 1933). The platelets observed in excess over the usually accepted nor-

mal number are, however, of the "micro" type, and it has been demonstrated that most of these forms are artefacts (Hartman), formed either by contact of the blood with tissue juices in the presence of Tyrode solution (Preiss) or by precipitation of the calcium chloride of the solution when this is in contact with the alkali of the glass (Lampert, 1931 [*b*]). Lampert (1931 [*a*]) used this solution extensively and eventually (1931 [*b*]) came to the conclusion that the *kleinen Thrombozyten* were crystals of insoluble calcium carbonate.

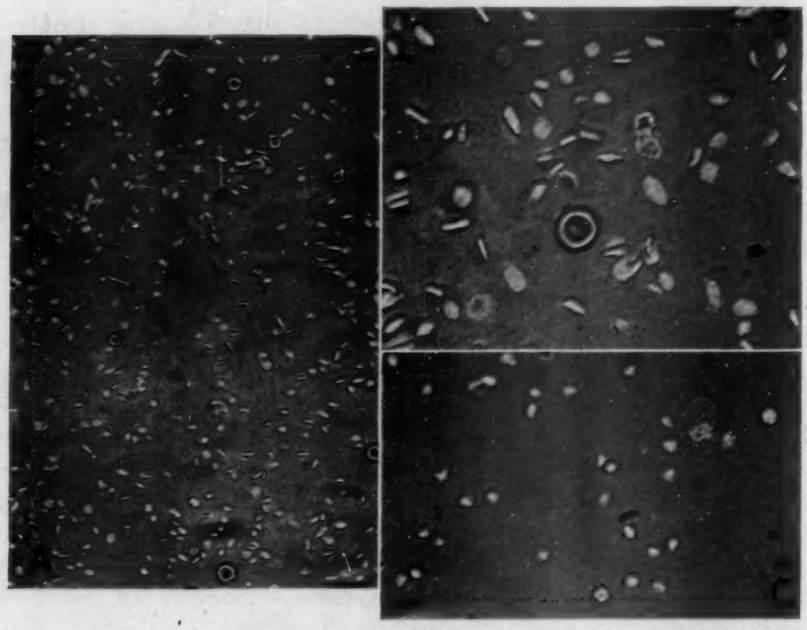
Pipets and syringes of special shape (Schenk; Kristenson, 1922 and 1928; Jurgens) and composition (Lampert, 1931 [*a*]) have been employed; although useful in the hands of their originators, they offer little advantage over the standard types available. Counting chambers such as that of Helber (depth, 0.02 mm.; cover glass thickness, 0.1 mm.), the device proposed by Bernsdorff or the Petroff-Hausser counting chamber for bacteria (depth, 0.02 mm.; cover glass thickness, 0.18 mm.) (Baldwin, Petroff and Gardner) are useful but do not seem strictly necessary. The standard Burker, Thoma-Zeiss and Levy-Hausser counting chambers (depth, 0.1 mm.) and the Thoma diluting pipets, if properly calibrated and certified, seem entirely adequate.

A magnification of from ×400 to ×600 with dry objectives seems best. Higher magnifications may be obtained with chambers less than 0.1 mm. in depth and with use of thin cover glasses. Oil immersion objectives often disturb the cubic content of the chamber, and the use of low power objectives (Casey and Helmer) is not advisable. While identifying platelets, the observer should have the fingers of one hand on the fine adjustment to obtain the critical focusing that reveals the characteristic highly refractile, silver chip appearance of the platelet. Only forms from 1 to 3 microns or longer in size, rod or comma-like if seen sidewards, and thin, translucent and disklike if flat on the floor of the counting chamber, should be counted. Granules 0.8 micron in diameter or smaller, jerkily moving about more or less actively, globules of oil, irregularly shaped débris floating on the upper layers of the fluid, strings of cocci and other minute objects may be distinguished from platelets after a little practice. The error of counting too few platelets may be equaled only by the error of counting every particle in the field as a platelet.

Some workers believe that there may be an advantage in expressing the number of platelets per cubic millimeter as of plasma instead of as of whole blood (Thomsen, 1923; Windfeld; Jurgens). For example, when the total volume of blood is decreased, as in chronic anemias, it may be better to know the platelet content of the plasma instead of that of the whole blood. Furthermore, if, as is generally supposed, the volume of plasma remains approximately the same during fluctua-

tions in the total corpuscular volume, the number of platelets per cubic millimeter of plasma furnishes a more reliable indication of their total number. The irregular distribution of platelets in the circulation takes much force out of this argument.

Many of the suggested precautions are often useful under certain conditions. Platelets may be counted, however, just as easily as erythrocytes if coagulation of the blood is effectively prevented and the platelets are fixed as the blood is withdrawn, without contact with the tissues or any wettable surfaces. Thus fixed, normal platelets show very little change at room temperature for twenty-four hours (*A* in the figure).



A, well preserved platelets from the blood of a dog; × 450. The platelets were suspended in citrated solution of formaldehyde, and the preparation had been standing for twenty-four hours when the photograph was taken; *B*, the same; × 900. *C*, platelets from human blood, collected into 3.2 per cent sodium citrate solution and allowed to stand for two hours; × 900. Nearly every platelet is undergoing swelling and formation of vesicular excrescences, the first stage of disintegration.

It is the initial stage of alteration in the platelet that renders it so strongly adhesive and agglutinable. Rapidly fixed platelets preserve their size and shape and adhere very little to glass surfaces or to each other. Therefore, a diluting fluid for total and differential platelet counting should: (1) effectively prevent coagulation of the blood and changes in the platelets; (2) fix the platelets as the blood is being removed from the vessels; (3) be isotonic with the blood and cause

no precipitates with the plasma or the glassware, and (4) be of simple composition and of easy preservation. Of the fluids available for platelet counting, the Rees-Ecker solution (of which the solution to be described is only a slight modification) seems to have all the necessary ingredients in the best combination. A stain is necessary only until one has gained experience in recognizing platelets. The following solution has been found to fulfil the requirements: sodium citrate, 3.8 Gm.; solution of formaldehyde (neutral, 40 per cent), 0.2 cc.; distilled water, 100 cc. If a stain is desired, add 0.05 Gm. of brilliant cresyl blue. Keep in a well stoppered bottle in an icebox, filter each time just before using, perform blank counts on the solution at intervals and look for bacteria, molds and platelet-like bodies. Use glass stoppers for all containers. The use of cotton or lint should be avoided.

For the enumeration of platelets in venous or arterial blood of man or the larger laboratory animals (dog, rabbit, sheep and others) a technic was developed which seems to fulfil the requirements outlined. It allows making repeated observations on the numbers and morphology of intact platelets as well as of erythrocytes and leukocytes, on the volume of packed cells and on stained smears of blood.

AUTHOR'S TECHNIC

The apparatus consists of: a syringe of the type used in making the tuberculin tests, having a capacity of 0.5 cc. and graduated in tenths or five hundredths of a cubic centimeter; vials of 2 cc. capacity, containing a glass bead and having glass stoppers ground to fit; an automatic shaker; pipet and counting chambers certified by the United States Bureau of Standards. The solution is kept at from 2 to 4 C. after being filtered. It is placed in small test tubes and sterilized in the autoclave along with tubes containing distilled water, loss in fluid in the solution during sterilization being made up with the distilled water. The needles, syringes and vials are sterilized by dry heat. Counting chambers and cover slips are boiled once weekly for two hours in distilled water containing a small amount of sodium bicarbonate; pipets are cleaned by the usual changes of water, ether and alcohol.

The method is as follows: Aspirate 0.4 cc. of the diluting fluid into the syringe and dry the tip with a sterile gauze stopper. Adjust a sterile no. 27 gage needle to the tip snugly, to prevent any ingress of air. Then carefully push the solution up the shaft of the needle until it just appears at the lumen. For needles of the size specified, 0.03 cc. of solution is usually needed to fill the shaft of the needle. This, therefore, will displace the piston downward. Transfer to the sterile vial enough of the solution to bring the piston down to coincide with one of the rulings on the barrel. Enter the vein after distending it slightly by application of a little pressure above the site of puncture. Once the needle is in the vein, remove the pressure, and allow approximately five seconds to elapse before withdrawing blood. Slowly withdraw exactly 0.1 cc. of blood into the syringe. Then place the tip of the needle under the level of the solution in the vial, and empty the contents of the syringe into it, aspirating and emptying the solution and blood slowly, without bubbling, three or four times to insure thorough mixing.

Stopper the vial and place it in an automatic shaker, where it is to be rhythmically agitated for at least five minutes. The speed of the motor should be so adjusted by a rheostat that the shaker gives between 80 and 100 strokes a minute. From the shaken mixture make a 1:100 dilution, using a diluting pipet and the same diluting fluid. Submit this dilution also to shaking, then place a drop of it in two counting chambers, and count the erythrocytes in 160 of the small squares of each chamber; multiply the average of the two counts by 12,500. Allow the preparation to stand in a moist chamber for at least twenty minutes before counting the platelets. Count all platelets in 400 small squares (or the entire finely ruled area of each chamber) and multiply the average of the two counts by 5,000.

In man the basilic veins in the forearm are used for venous platelet counts; in dogs, the leg, arm or ear veins, and in rabbits, the ear veins. For platelet counts on arterial blood in man the radial artery is the most accessible; the brachial artery, at the elbow, may also be used; in dogs the femoral artery, along the thigh, is the most accessible. Counts done at the same time on blood from various channels are done preferably in this order: (1) venous blood; (2) arterial blood; (3) cutaneous blood (Tocantins, 1936 [*f*]). Whenever the syringe is used for making dilutions, it is essential for the operator to place himself and so arrange the arm of the subject that the syringe is held horizontally. This insures easy reading of the marks on the syringe without making errors because of parallax, and insures detection of the moment when the artery is entered, for the pressure in the vessel will overcome the column of solution in the syringe if the latter is held horizontally. The syringe should be so turned that the graduation lines are on each side of, and not over, the piston. Measurements are made by adjusting the end of the piston along the same line as graduation markings on the side, thus preventing parallactic errors, which are unavoidable when the end of the piston is adjusted under the ruling. For a better visualization of the end of the piston and the markings, a special magnifying device to be described in a later publication may be used.

If the platelets are above 1,000,000 per cubic millimeter, dilutions higher than 1:5 are used to reduce the chances of agglutination. Windfeld's contention that high dilutions of blood yield correspondingly higher counts because of fragmentation of platelets applies only when no fixatives are used. If there is reason to expect a low platelet count, the blood is diluted 1:2 in the syringe instead of 1:5, and special precautions should be taken with the diluting fluids. If the platelets in the chamber are few, the presence of any extraneous matter in the diluting solution will often cause the most experienced technician to include them in the count if they resemble platelets. The most scrupulous cleaning of the glassware and filtering of the solutions will leave a very small amount of particles in suspension. When the platelet count is normal, these particles usually do not greatly alter the result, since in any solution they should not be present in greater concentration than 5 particles per tenth of a cubic millimeter of the solution. When few or no platelets are present, however, they may look enough like platelets to cause the observer to count them as such, thereby greatly exaggerating the platelet count. If the diluting solution includes a dye like brilliant cresyl blue, this difficulty is increased. For this reason, with every series of platelet counts, a blank count on the filtered solutions should always be done and the result subtracted from the platelet count if the solution contains only 5 or less platelet-like bodies per tenth of a cubic millimeter. This practice not only increases the accuracy of the count but gives also a frequent check-up on the solutions. The total number of platelets is calculated by subtracting the number of platelets counted

in the blood dilution preparation (*A*) from the number of platelet-like bodies per tenth of a cubic millimeter in the diluting solution (*B*) according to this formula:

$$\frac{(A\text{-}B) \times \text{dilution} \times 4{,}000}{400} = \text{number of platelets per cubic millimeter of blood.}$$

In thrombopenic bloods, platelets are further searched for in a smear of blood made from the mixture and stained with Wright or Jenner-Giemsa stain; after the erythrocytes have settled in the vial, a loopful of the supernatant plasma is examined for the presence of platelets as a hanging drop. Occasionally (when the count is very low) the drop of plasma may be introduced without further dilution into the counting chamber, the platelets counted and the total number of platelets per cubic millimeter of *plasma* obtained by multiplying the total number counted in the chamber by the dilution × 10.

In this manner one may combine many of the desirable features of the Fonio, Thomsen and Teoumine methods.

MORPHOLOGIC STUDY

Study of Wet Specimens.—This may be carried out in a drop of plasma removed from the diluted blood obtained for platelet counting by my method. The plasma is placed either between a cover slip and slide or in a hanging drop slide with a ring of petrolatum around it to prevent evaporation (figure). For dark field work, a thin slide and cover slip must be used. For further details consult Aynaud and Jeantet, also Ferguson (1934 [*a*]).

To observe the various phases of the transformation and disintegration of platelets the following method is used:

Collect blood into a syringe containing 2 per cent sodium citrate in physiologic solution of sodium chloride (1:1). Place the mixture in a paraffin-coated tube and centrifugate for one minute at 2,000 revolutions per minute. Place a drop of the platelet-rich supernatant fluid in each of four or five cover slips; ring each of these with petrolatum and place them in a moist chamber at room temperature. At one hour intervals take one of the cover slips, add a very small drop of a filtered 0.5 per cent aqueous solution of brilliant cresyl blue and invert the cover slip over a clean slide.

The following method is useful in a study of the unaltered platelets in the plasma of an animal:

Collect blood directly from an artery or a vein immersed under the surface of a 2 per cent solution of sodium citrate in physiologic solution of sodium chloride. If it is desired not to use anticoagulants, collect the blood with paraffin-coated syringes and needles, place in cold paraffin-coated vessels, tightly stoppered, and centrifugate at low speed for from five to ten minutes. Then, with a fine paraffin-coated pipet remove a drop, place it on a clean cover slip, ring the cover slip with petrolatum and invert it over a hanging drop slide (Tocantins, 1936 [*b*]).

This technic may be applied to blood without centrifugation but is specially useful when one wishes to study slowly coagulating plasmas,

such as those of hemophilic, jaundiced and, in some instances, thrombopenic patients and of animals, such as the horse or donkey, the blood of which coagulates more slowly than that of man or dog. If body temperature is required, the slide may be placed in a warm stage at 37 C.

Differential counting of platelets with respect to size is performed after this method:

Measure, with the aid of a micrometer eyepiece, along their greatest diameter all platelets in the 400 small squares of the counting chamber as they rest on the floor of the latter. Calculate the total number of each type per cubic millimeter of blood by multiplying the number counted in each group by the factor used to determine the total number of platelets per cubic millimeter. Platelets may be grouped into those measuring 1, 2, 3, 4 and more microns along their greatest length, and frequency curves may be built from the figures. Consult also Reimann.

Vital staining of platelets has been done by a number of investigators. Levaditi, also Rosin and Bibergeil, applied vital red and brilliant cresyl blue to the study of platelets. Pagniez evaporated a thin film of a solution of brilliant cresyl blue in absolute alcohol on a clean slide, placed a drop of blood over the thin film of the stain, covered it with a cover slip, and examined the preparation thus made. Aynaud (1909) used a 1:25,000 solution of neutral red for vital staining of citrated platelets. Mitochondria may be demonstrated in platelets by supravital staining with Janus green. Cytologists in general recognize the material in leukocytes stained with Janus green as being mitochondria. By means of this stain, Ma demonstrated mitochondria in platelets.

Ma Method: Use clean, grease-free glassware. Make a saturated solution of Janus green in distilled water. Prepare a film of dye on a slide with a dilution of the saturated solution—from 5 to 8 drops to 10 cc. of distilled water. Place a small drop of blood in the center of a clean cover slip and drop the cover slip immediately over the prepared slide. Seal the blood spread with petrolatum and observe the preparation in a warm stage. Platelets will usually show from 4 to 8 small mitochondria. A material that is stainable with neutral red may also be demonstrated: Prepare a dye film with a mixture of a saturated solution of Janus green (5 drops), neutral red (5 cc.) and distilled water (10 cc.). The blood platelets of rats show approximately equal numbers of bodies stained with Janus green and neutral red, respectively. Like reticulocytes and erythrocytes, platelets contain mitochondria and Golgi lipoid material (Ma).

Fresh supravital staining preparations may be made by Sabin's technic. With a neutral red-Janus green combination, the platelets show two types of granules; those of one type occur in clumps and are stained with neutral red, and those of the other occur as separate particles and are stained with Janus green; neither type of granules, however, show any brownian motion. The method is particularly adaptable to the demonstration of megakaryocytes in preparations of blood,

for the usual method of preparing dry smears damages these cells beyond recognition.

Deetjen (1901) Methods: 1. Prepare a 1 per cent agar solution in a 0.6 per cent sodium chloride solution and filter it. Warm a clean slide, allow a few drops of the solution to flow on the slide so as to make a thin film, allow the slide to cool, and place a drop of blood on the surface of the film and a cover slip over it. The preparation may then be fixed with fumes of osmic acid. To demonstrate movements in the platelets, add 0.6 Gm. of sodium metaphosphate and 0.3 Gm. of dipotassium phosphate to the agar solution just described (Deetjen).

In blood placed in these agar films Deetjen saw the platelets moving as late as twenty-four hours after collection, when the preparation was kept at room temperature. Their action appeared to be greatest at body temperature and at 40 C. Too high a concentration of salt in the solution inhibits the movement, and any concentration less than 0.4 per cent is undesirable. The best concentration of the sodium metaphosphate is between 0.4 and 1 per cent. The preparation may be fixed at any time by letting 1 per cent osmic acid run under the cover slip. From three to five minutes are sufficient to allow for fixation of the cells. The cover slip is then removed and the preparation stained with any of the ordinary stains. Deetjen recommends hematoxylin and eosin. Deetjen's solution is prepared as follows: Take 5 Gm. of agar-agar and 500 cc. of distilled water, heat for half an hour and filter while hot. To each 100 cc. of filtrate add 0.6 Gm. of sodium chloride, from 6 to 8 cc. of 10 per cent sodium metaphosphate solution and 0.5 cc. of 10 per cent dipotassium phosphate solution. This should not be heated to get the salt to dissolve; after the addition of the sodium metaphosphate, the agar solution becomes clearer. This solution should not be cloudy before it is used. Pour some of it on a clean slide and cool; cut a piece 2 mm. wide and put it over the finger or wherever the blood is to be obtained. Make the puncture of the skin through the agar and invert it over the slide.

2. To isolate platelets in small amounts from the blood, Deetjen used the following technic: Place a drop of blood on a clean cover slip and invert this on a slide on which two thin capillary tubes have been placed parallel to each other. This allows a thin film to form between the cover slip and the slide. With a pipet filled with salt solution play a fine stream against one side of the film and hold a filter paper on the other side. This will wash out most of the red cells and white cells and leave the platelets in the preparation. The glassware must be free from any dirt or grease. Deetjen (1909) used quartz glass slides and square cover slips, 0.2 mm. thick and 18 mm. long, made from molten quartz.

By such a technic only grossly altered platelets may be seen.

Study of Dry Specimens.—Results differ, depending on whether the blood is allowed to dry before fixation or is fixed before it dries. They differ further if fixation is accomplished before the smear is made. Platelets are least altered if they are fixed as soon after collection as possible and before they are put through any mechanical manipulations. Most stains used today combine fixing and staining processes in one.

A differential count of platelets in the dry smear can yield only an approximate and often erroneous idea of the relative numbers of the various forms. Indeed, the platelet, for reasons already exposed, is susceptible of being changed by the commonly used technic of examina-

tion of the blood more than any of the other morphologic elements of the blood. The study of the tinctorial characteristics of the platelet has been of use mostly in throwing light on its relation to other cells and on the question as to the presence of a nucleus in the platelet. Aynaud (1909) expressed the belief that the staining reactions of the platelet are due to amphophilic material rather than to two substances, one basophilic and the other acidophilic. Cajal used reduced silver nitrate to stain platelets. Platelets may be well demonstrated by any of the Romanowsky stains, and they show fairly good differentiation of the granules with hematein, pyronine and toluidine blue if the smears are allowed to be stained for a prolonged period with these dyes. They do not stain with methyl green, and they absorb the pyronine when treated with Pappenheim's methyl green-pyronine combination (Downey).

1. Downey Method: Fix the smear in a mixture of equal parts of 2 per cent osmic acid, saturated solution of iodine in 100 per cent alcohol and solution of formaldehyde U.S.P. by allowing the moist smear to be exposed to the fumes of this mixture for from twenty to thirty seconds. Allow it to stand over night before staining in Giemsa liquid.

2. Aynaud (1909) Method: Place a drop of fresh oxalate-prepared or citrate-prepared plasma containing platelets on a clean side and carefully make a thin smear from it. Fix in absolute alcohol (one hour) or in Flemming's mixture (ten minutes for fixation, washing in running water for twenty-four hours). Stain for from one to two hours with Giemsa stain diluted to 1.5 per cent. Wash in running water for one minute and treat the preparation with a 5 per cent solution of tannin for fifty seconds. Wash thoroughly and dry. Various forms of platelets are thus obtained.

It is preferable to fix the blood before making the smear. Aynaud (1909) used the following method: With a paraffin-covered pipet obtain a few drops of platelet-rich plasma from citrate or oxalate-prepared blood and allow them to fall into 10 cc. of 1 per cent osmic acid solution, shake and centrifugate. The fixed platelets sediment rapidly. Remove the sediment, spread on clean slides, dry in the air, wash for twenty-four hours in running water and stain as outlined. Solution of formaldehyde may be used instead of osmic acid and the platelets separated by fractional centrifugation if whole blood is used.

Platelets fixed directly in solution of formaldehyde present a very homogeneous appearance. Staining does not disclose any sharp separation between cytoplasm and granules. The entire platelet appears made up of a homogeneous mass with fine granules diffused through it.

3. Jenner-Giemsa Method: Fix the film in methyl alcohol for one minute. Stain with the Jenner stain (2 drops to 1 cc. of distilled water) for five minutes. Wash in distilled water. Stain with Giemsa solution (1 drop to 1 cc. of distilled water) for twenty minutes. Wash in distilled water. This stain may be applied to the blood films even after they have been mixed with formaldehyde-citrate solution; the small amount of solution of formaldehyde in this mixture does not affect the results.

4. Corradetti Method of Differential Staining of Platelets and Megakaryocytes: Fix the smears from the blood or bone marrow in ethyl alcohol (95 per cent)

for from twenty-four to forty-eight hours. Wash in water for a few minutes and place in a normal solution of hydrochloric acid at 60 C. for from forty-five to sixty minutes. Wash again in water, carefully so as not to remove the smear; allow the preparation to dry. Stain with stock Giemsa solution for one hour; stain with a diluted solution of Giemsa stain (1 drop of stock Giemsa solution per cubic centimeter of distilled water). This method destroys the erythrocytes and leukocytes with the exception of the granules of eosinophils. The platelets and the cytoplasm of megakaryocytes are preserved and show the azurophil granulations. The nuclei are not stained.

The platelets in the spleen may be studied by impressions of the organ on glass slides or by teasing the tissue in citrate solution. The platelets are found in rows or in small loose masses, located particularly in the sinuses, sometimes along the medullary cords but not in the malpighian follicles. Consult Foa and Carbone, also Foa; in the article by Foa, a colored plate illustrating the location of the platelets may be found.

For other methods of staining and of morphologic study of platelets consult: Bizzozero (1891); Rabl; Eisen; Dekhuzsen; Puchberger; Weidenreich; Aynaud (1909); Degkwitz; Riess; Sabrazes; Oselladore (1926); van Walsem.

5. Aynaud-Pettit Method of Demonstrating Platelets in Chicken Blood: Obtain the blood directly from the vessels through and into paraffin-coated tubes, avoiding all contact with tissues. Centrifugate at slow speed for from five to ten minutes. Then, with a paraffin-coated pipet, place a few drops of the plasma in the following solution: 10 per cent sodium citrate solution, 9 volumes, and a 40 per cent solution of formaldehyde, 1 volume, or in this one: 10 per cent aqueous solution of sodium citrate, 1 part, and 2 per cent aqueous solution of osmic acid, 1 part. Centrifugate at high speed for from ten to fifteen minutes. Make a smear from the sediment; allow it to dry for several hours and stain with Giemsa, Leishman or Wright stain.

For the study and counting of the thrombocytes of birds, it is, of course, necessary to stain the thrombocytes with a differentiating stain such as one of the Romanowsky mixtures, for it is impossible to differentiate between thrombocytes and the other elements of the blood in wet, unstained specimens (Cossali). For methods of counting the thrombocytes of birds consult: Gohs; Shaw; Magath and Higgins. For an indirect method of counting consult Wiseman.

STUDY OF PLATELETS AND MEGAKARYOCYTES IN SECTIONS OF FIXED TISSUES

The organs and pieces of tissue must be handled very gently. If lung tissue is to be studied, the organ should not be allowed to collapse before removal. The trachea is clamped before opening the thorax; the lung is removed while still expanded and is completely immersed in the fixing reagent. Cutting should be done with unusually sharp

knives to avoid excessive pressure on the tissue, for much of the evidence sought depends on changes in the form of the cells. Special precautions must be taken with the sections to avoid displacing the contents of blood vessels, where many of the changes in the platelets and megakaryocytes are to be observed. The thin sections should be cut in series and laid, well stretched, over the slide. The albumin-glycerin solution should be placed on the slide first and then the section well flattened over it. Dehydration with acetone should be carried out rapidly and over a cold plate to avoid condensation of water on the section from rapid evaporation of the acetone. It is best to allow the acetone or the alcohol to run over the section gently from the side before the section is immersed in the liquid. The excellence of tissue sections stained with Wright and Cesaris-Demel solutions may be explained by the minute care taken in their preparation.

Fixation and Staining.—Methyl alcohol, formaldehyde and mercury bichloride are the most commonly used fixatives. Zenker's fluid is not recommended. Fixation with methyl alcohol permits ultimate staining of the azurophil granules of platelets and megakaryocytes without resorting to an acid for decolorization.

1. Ogata Method: Fix tissues with a 10 per cent dilution of solution of formaldehyde U. S. P. at 36 C. for from two to three hours. Cut sections 2 mm. thick and fix for twenty-four hours in the same solution. Fix for twelve hours in Müller's fluid. Stain sections 4 microns thick by the azur II eosin method of Schridde.

Gaspar modified Ogata's technic by treating the formaldehyde-Müller fixed material with 5 per cent nitric acid for twenty-four hours and then with 5 per cent disodic sulfate solution for from one to two days before embedding. The acid destroys the chromatin of the nuclei but does not affect the granules of the megakaryocytes and platelets, which stand out clearly.

2. Cesaris-Demel Technic: Fix small pieces of the organ, which should have been removed as soon after death of the animal as possible, by the Ogata method. Embed the specimen in paraffin and cut sections between 5 and 7 microns thick. Stain for from seventeen to twenty-four hours in distilled water, 100 cc., plus Giemsa solution (Ciba), 30 drops. Dehydrate with neutral acetone, clear with xylene and mount in pure cedar oil, as balsam decolorizes the section rapidly. The granules of the platelets and the cytoplasm of the megakaryocytes are colored wine red, and the hyalomere, a light pink, which contrasts with the azure of the other elements.

Nicoletti advised adding to each 100 cc. of the diluted Giemsa solution from 0.1 to 0.3 cc. of a 10 per cent solution of calcium chloride when the water used for dilution does not give satisfactory results. He believed that calcium ions exert a distinct influence on the elective staining of the azurophilic granules of the platelets and megakaryocytes.

3. Wright Technic: The author's own description, with minor revisions, is followed: The material should be obtained immediately after death or taken from the living animal. For fixation, methyl alcohol, formaldehyde or a saturated solution of mercury bichloride in a 0.9 per cent solution of sodium chloride may

be used. Methyl alcohol is not now recommended for fixation. Formaldehyde should not be allowed to act longer than forty-eight hours. The method is not applicable to material fixed in Zenker's fluid. The tissue is dehydrated by alcohol followed by acetone, cleared in thick oil of cedar followed by xylene, and embedded in paraffin. The sections should not be more than 4 microns thick. Crystals of mercury bichloride in the sections are to be removed by treating the sections with Gram's solution of iodine and alcohol. The sections are stained while affixed to the slide by Meyer's glycerin-albumin mixture. The staining fluid is composed of a mixture of a solution of modified, or polychromatized, methylene blue, 3 parts, and a 0.2 per cent solution of eosin in pure methyl alcohol, 10 parts. It is permanent if it is kept in a well stoppered bottle so that evaporation is prevented. The staining, clearing and mounting are carried out as follows: (*a*) Mix equal parts of the staining fluid and distilled water in a small wine glass and immediately pour the staining mixture on the slide. The measuring is conveniently done by means of a small pipet provided with a rubber bulb. At least 2 cc. of the freshly diluted staining fluid is thus spread over the slide, which should be supported on some object in such a way as to prevent the fluid from running off. The spreading out of the fluid in a layer is important because it facilitates the evaporation of the alcohol whereby the staining elements slowly precipitate out of solution and, while doing so, stain the tissue elements. This precipitate appears as a yellowish metallic scum which slowly forms on the surface of the mixture. The diluted staining fluid is allowed to act for about ten minutes, and then the preparation is immediately washed in water. The exact time required for the best results has to be determined for each batch of the staining fluid. The proper staining of the preparation may be judged by examining it by a yellowish artificial light under low magnifying power after pouring back the diluted staining fluid into the wine glass. The stain is to be regarded as sufficiently intense and the staining process stopped by washing the preparation in water when the cytoplasm of the giant cells has acquired a bright red color and the fibrils of the reticulum begin to take on a red color also. If the staining is found not sufficiently intense, the diluted staining fluid is poured back on the preparation and allowed to act longer. Overstaining and the formation of a dark red granular precipitate on the preparation occur if the diluted staining fluid is allowed to act longer than a certain time.

(*b*) Dehydrate in pure acetone. On account of the great volatility of acetone, some care is necessary to prevent drying of the preparation, which should be avoided.

(*c*) Clear in pure oil of turpentine.

(*d*) Mount in a thick solution of rosin (colophonium) in pure oil of turpentine. Before mounting the preparation, the superfluous turpentine should be carefully removed, because this reagent rapidly takes up water from the air and thus may cause the clouding of the preparation or the fading of the stain. The solution of rosin is made by saturating a quantity of turpentine with powdered rosin and keeping the filtered solution in the oven used for embedding in paraffin until it has evaporated to the required consistence. The use of acetone for dehydrating and of oil of turpentine for clearing the mounting is an important feature of the method, for these do not destroy the characteristic staining of the granules in the giant cells and platelets as do other similar reagents that have been tested (Wright).

Downey modified Wright's fixation mixture and adapted it for demonstration not only of megakaryocytes and platelets but also of the lymphocytes of the

marrow, spleen and lymph glands. For a fixative he used a 10 per cent dilution of solution of formaldehyde U.S.P. in physiologic solution of sodium chloride saturated with mercury bichloride, and for a stain he used Wright's, Giemsa's or Dominici's. To obtain the best results with the Wright stain, it is important that the fixing fluid contain mercury bichloride and that acetone be used for dehydrating, turpentine for clearing and turpentine damar for mounting (Downey).

4. LeSourd-Pagniez Technic: Fix pieces of tissue, as thin as possible, in Dominici's liquid (an iodized mixture of mercury bichloride and potassium bichromate) for between twelve and fifteen hours; embed in paraffin, and section very thinly. Apply diluted Giemsa stain in two stages: (*a*) Prepare a mixture of 15 cc. of distilled water with 5 drops of Giemsa stain and immerse the sections in this mixture for from twelve to fifteen hours; (*b*) prepare a mixture of distilled water, 15 cc., and Giemsa stain, 15 drops, and immerse the sections in this mixture for from four to five hours. Treat the sections afterward with the following mixtures of acetone and xylene: (*a*) acetone, 18 drops, and xylene, 2 drops; (*b*) acetone, 14 drops, and xylene, 6 drops; (*c*) acetone, 6 drops, and xylene, 14 drops. Allow each of these mixtures to fall, drop by drop, on the sections until good differentiation has been acquired. Wash with pure xylene and mount in balsam. Essential points for success in the staining are the renewal of the staining bath and the substitution of acetone for alcohol in the dehydration. Platelets are stained reddish violet very much as in blood smears treated with Giemsa stain. The protoplasm is only faintly visible. The platelets in the spleen are found in little groups outside of the follicles in the rabbit, guinea-pig, cat and man (Lesourd and Pagniez, 1911 [*b*]).

For details of the methods of demonstrating platelets and megakaryocytes in tissue sections consult: Dominici, Bunting, Brown, Bernhardt, Ferrata and Negreiros-Rinaldi, Di Guglielmo, Pianese, Jolly, Frank and Seeliger, Seeliger, Kaufman, Gunn, and Howell and Donehue.

VOLUMETRIC MEASUREMENT

Only an approximate idea of the total volume of platelets in the blood may be obtained from determinations of the percental volume as currently done. Such determinations may be expected to give an indication only of the volume of platelets in the blood in the vessel from which the blood was collected, for the number of platelets varies in different vessels and regions of the body. The technic of Van Allen may be modified so as to give the average volume of each platelet and, knowing the number of platelets per cubic millimeter in a particular sample of blood, one may calculate the percental volume in this sample. In general, the objections to Van Allen's method (which seems the best available) are principally that some leukocytes may be packed down with the platelets, thus increasing the volume of the platelets, and, since (if Van Allen's solution is used) the platelets are not fixed, their volume may change on withdrawal of the blood and standing of the specimen for any length of time.

Modified Van Allen Method.—The following apparatus is needed: a thrombocytocrit,[1] a 10 cc. record syringe and a centrifuge capable of 3,500 revolutions per minute.

Draw 6 cc. of a filtered solution containing sodium citrate, 3.8 per cent, and solution of formaldehyde U.S.P., 0.1 per cent, into a record syringe. With a sterile needle, gage between 20 and 22, withdraw 4 cc. of blood rapidly, without stasis, into the solution. Remove the needle and tilt the syringe two or three times to insure good mixing. Place the diluted blood in a paraffin-coated centrifuge tube of 15 cc. capacity and mix the contents gently for five minutes in an automatic shaker. Perform a platelet count on this mixture, making allowance in the calculation for the original dilution used. Centrifugate at 1,000 revolutions per minute for five minutes if the blood is of normal erythrocyte content; with blood containing from one half to one third the normal number of erythrocytes, centrifugation for from two to three minutes is sufficient for separating the red and white cells. With a paraffin-coated pipet, remove approximately 2.5 cc. of the platelet-rich fluid to another paraffin-coated tube. This is to be corked and shaken gently for five minutes in an automatic shaker. The second platelet count is done on this suspension. Draw exactly 2 cc. of the suspension into the thrombocytocrit by applying a mouth suction tube over its upper end and dipping the graduated end into the measured solution. Before beginning the aspiration, place the ring of the metal clip over the upper end of the thrombocytocrit and allow the metal bucket with its rubber base to hang loose. Immediately after all the fluid has been aspirated, apply the metal bucket to the bottom of the graduated tube. Then place the thrombocytocrit in a centrifuge cup and balance it with another thrombocytocrit filled with 2 cc. of water as a counterweight. Centrifugate at 3,500 revolutions per minute for one and a half hours, at which time the capillary lumen of the thrombocytocrit is found partly filled with a white sediment, the supernatant fluid being clear. Read the actual amount of white sediment directly from the scale on the tube, in cubic millimeters. Divide this figure by the *total number* of platelets in the centrifugated plasma. The result gives the mean volume for the individual platelet. To obtain the volume of the platelets per hundred cubic millimeters of blood (percental or thrombocytocrit volume) of the sample collected, it is necessary only to multiply the volume of the individual platelet by the number of platelets per hundred cubic millimeters of blood. For example:

Number of platelets per cubic millimeter of blood: 300,000

Volume of packed platelets in cubic millimeters: 13

Platelet count per cubic millimeter of centrifugated plasma: 530,000

Amount of centrifugated plasma: 2 cc.

Total number of platelets in the centrifugated plasma: $2 \times 530{,}000{,}000 = 1{,}060{,}000{,}000$ platelets

Mean platelet volume $= \frac{13}{1{,}060{,}000{,}000} = 0.000{,}000{,}012$ cubic millimeter $= 12$ cubic microns

The percental volume of the platelets in whole blood (thrombocytocrit) is calculated from the mean platelet volume (12 cubic microns) multiplied by the number of platelets per cubic millimeter of blood (first platelet count) multiplied by 100:

$$0.000{,}000{,}012 \times 300{,}000 \times 100 = 0.36$$

Percental volume of platelets $= 0.36$ per cent

1. The Van Allen thrombocytocrit may be obtained from the A. H. Thomas Company, Philadelphia.

A general idea of the percental volume of platelets in man may be obtained by dividing the figure expressing the number of platelets per cubic millimeter of blood by 1,000,000. For example: If the number of platelets per cubic millimeter of blood is 260,000, the percental volume of platelets is 0.26 per cent. This method of calculation does not apply, of course, when the platelets show great variations in size.

Centrifugation of the plasma should be carried out until complete packing of the platelets has taken place. The time required varies with the type of centrifuge used. If a few erythrocytes are present in the platelet-rich plasma, they are packed as a distinct layer on the bottom of the tube and often make easier the reading of the volume of packed platelets.

For further details consult: Van Allen, Endres, Horwitz (1931), Nygaard and Duxbury.

ISOLATING PLATELETS FROM BLOOD

The anticoagulants most commonly employed for this purpose are the citrates and the oxalates. To keep the platelets intact, the collection of blood should be as rapid as possible and any contact with wettable surface avoided. If it is desired to dilute the blood as little as possible, concentrated solutions of anticoagulant (20 per cent sodium citrate or 25 per cent potassium oxalate) may be used, but unless the blood is collected rapidly and mixed with the anticoagulant at once, many platelets are altered in these solutions.

In Man.—Place the amount of anticoagulant solution necessary to keep the blood from clotting in the syringe and wet the sides of the syringe thoroughly with it. If the solution is sterile, wet the interior of the needle as well. It is advisable to use an excess of an isotonic solution of the anticoagulant and a syringe of 50 cc. capacity. With a sharp needle of short shank and wide bore, puncture the vein and draw the blood swiftly into the solution. Collect approximately 30 cc., empty the contents into two paraffin-coated, round bottom centrifuge tubes in each of which there has been placed approximately 25 cc. of a mixture of citrate (or oxalate) and physiologic solution of sodium chloride. Mix thoroughly by pouring from one paraffin-coated tube into the other. Centrifugate at about 1,000 revolutions per minute for from four to five minutes. Pipet off the supernatant fluid, which is of a light pink color, into paraffin-coated, conical bottom centrifuge tubes and centrifugate for one hour at about 2,000 revolutions per minute. Pipet off the supernatant fluid and discard it. The accumulated sediment appears distributed in two layers: a thin layer of red blood cells and, superimposed on it, a thick white layer of platelets, among which are mixed a few white and red blood cells. Cover the sediment gently with approximately 10 cc. of cold physiologic solution of sodium chloride. By applying suction through a glass pipet (capillary tubing drawn out to a tip measuring about 1 mm. in internal diameter), remove the white layer carefully, beginning with the central portion and gradually working toward the periphery and more deeply toward the packed layer of red blood cells. Place the mixture of salt solution and platelets into a 15 cc. conical bottom glass tube and emulsify gently by aspirating and expelling it with a dropping rubber bulb pipet. Avoid bubbling. After the solution is free from coarse floating particles and has an even pale blue translucent appearance, centrifugate it again for one hour at 2,000 revolutions per minute or until the supernatant fluid is clear. After the sediment is again packed, remove the supernatant fluid,

replace it with cold physiologic solution of sodium chloride and pipet off the white layer of platelets into another 15 cc. centrifuge tube; repeat the gentle emulsification and centrifugation. At least three centrifugations of the emulsified sediment are usually necessary to rid it of most of the white and red blood cells. Frequent microscopic observations of the emulsion should be made to make sure that the white and red blood cells are being removed. In pipetting off the white layer, no attempt should be made to remove it entirely, down to the level of the red blood cells; if a white film is left over the packed red cells, most of the white cells will have been eliminated. By this process approximately two thirds of the collected platelets are isolated.

The amount of blood collected may be adjusted, depending on the purpose for which the platelets will be used. Greater amounts may be isolated by having several syringes loaded with the anticoagulant solution and switching from one to another, the needle being left in place until the desired amount of blood is collected.

All glassware must be scrupulously clean, the solutions filtered and cold. All contact with cotton or thready material must be avoided; such material should not be used as stoppers for the bottles containing the solution. The collection of blood should be made early in the morning to allow sufficient time for the various steps during the rest of the day. The platelet emulsion, while not in use, should be kept in the ice chest at a temperature of approximately 5 C.

In Animals.—Platelets may be isolated from the blood of animals in a similar manner, the blood being collected by puncture of the heart or jugular vein in the unanesthetized animal or from the carotid artery. I have used cardiac puncture almost exclusively in collecting platelets from dogs, guinea-pigs and rabbits. From a dog weighing approximately 15 kilograms 200 cc. of blood may be collected from the heart without any disturbing symptoms. The puncture is made with the animal's head immobilized and the body firmly stretched and bound on a board. The syringes are loaded with the solution and laid by the side of the operator. Since the puncture is made almost vertically, a brake is placed on the piston to keep the solution from flowing out. The needle is left in the heart while syringes are being shifted. During a period of four years three dogs in the colony were used exclusively for this purpose. Two of these animals are still alive after sixty-two and ninety-six cardiac punctures, respectively; neither one has shown any circulatory disturbances, and each has given birth to three litters of pups during this period.

The modifications suggested by Hanausek for the collection of large amounts of platelets are useful. For other methods consult Endres (1927) and Horwitz (1930).

PREPARATION AND TESTING OF ANTIPLATELET SERUM

Preparation of Antiplatelet Serum.—The rabbit, guinea-pig and goat may be used; the rabbit seems the most desirable for the purpose. Six injections, spaced five days apart, of the platelets isolated from approximately 100 cc. of blood are sufficient to yield an antiserum of strong titer in the rabbit. Collection of the serum is done ten days after the last injection. Approximately 50 cc. of serum may be obtained from a rabbit weighing from 5 to 6 pounds (from 2.3 to 2.7 Kg.). Bleeding is best done from the carotid or the femoral arteries, with precautions to preserve sterility (Tocantins, 1936 [*c*]). For an improved method, consult Pagniez.

Determination of Titer.—1. By Agglutination: Prepare a suspension containing approximately 400,000 freshly isolated platelets per cubic millimeter and to fixed amounts of this suspension add fixed amounts of increasing salt solution dilutions of the serum. Incubate these mixtures and read the results at the end of four hours. Agglutination of platelets is judged not only by the amount of sediment in the tubes but also by the clearness imparted to the fluid. In tubes in which little or no agglutination of platelets has occurred, the fluid remains uniformly cloudy.

In a test of the agglutinating power of a serum or in any test in which the agglutination of platelets is involved, the following points should be observed: (*a*) Uniform platelet suspensions should be used (between 400,000 and 500,000 platelets per cubic millimeter). (*b*) The suspension should be *gently* shaken for about five minutes in an automatic shaker before it is distributed to the tubes. (*c*) Results are graded by observing the amount of precipitation and flocculation one and two hours after the tubes have been placed in the incubator at 37 C. The next day the reading is checked by noting the character and the amount of the precipitate and the clearness or the turbidity of the supernatant fluid as compared with the controls (suspensions of platelets alone). A heavy precipitate with a clear supernatant fluid is graded 4 plus; if the supernatant fluid is slightly turbid, 3 plus, and so on. If there is no agglutination of platelets in the first two hours, no flocculation and very little precipitation will be evident grossly during that interval. After twenty-four hours, however, about 70 per cent of the platelets of the suspension in the control tube will have settled, the supernatant fluid still remaining slightly turbid, thus affording a means of comparison with the other tubes.

2. By Ability of Serum to Inhibit Retraction of Blood Clots: Collect from 10 to 12 cc. of blood of known normal platelet content and place it in 1 cc. amounts in clean flat-bottom glass tubes, 8 mm. in internal diameter, into which there have been previously placed 0.1 cc. amounts of increasing dilutions of the serum—physiologic solution of sodium chloride being used as the diluent. Tilt the tubes carefully a few times to mix the contents and place in the incubator at 37 C. Examine the tubes at half-hour intervals for the first two hours and at the end of twenty-four hours. The titer is expressed in terms of the highest dilution that completely inhibits retraction of the clot. With each determination a control should be run with serum of known platelet agglutinating and intradermal purpurigenic potency. By comparison with the action of the control serum an approximate idea is obtained of the potency of the antiserum. The advantage of this method is that it is rapid and inexpensive.

3. By Reactions to Injection of Serum into Animals (Tocantins, 1936 [*c*]): Use two animals. With a 27 gage needle and a syringe graduated in hundredths of a cubic centimeter, inject 0.05 cc. of each of increasing dilutions of the serum, intradermally. Make two series of such injections, one on each side of the shaved abdomen, in the skin, in each animal, which must never previously have been given an injection of any serum. Read the results at the end of twenty-four hours. The purpurigenic titer of a serum is expressed in terms of the highest dilution that brings about a hemorrhagic patch in at least two of the four inoculations.

Of the three methods of evaluating the potency of an antiplatelet serum, the last one gives the most reliable results.

METHODS FOR STUDYING THE PROPERTIES OF PLATELETS

The properties most commonly studied are: (*a*) agglutination; (*b*) disintegration; (*c*) adhesion to and clumping about bacteria and foreign particles; (*d*) induction or acceleration of clotting; (*e*) induction of syneresis.

It is preferable to employ venous blood for these studies. An admixture of tissue juices markedly alters the plasma and platelets, thus affecting two of the properties most commonly tested: agglutination and disintegration. A distinction should be made between the properties of agglutination and disintegration. Platelets may agglutinate strongly and yet show little tendency to disintegrate, once agglutinated.

Methods of Determining the Rates of Platelet Agglutination and Disintegration.—The methods based on periodic platelet counts from standing blood have only very limited application. In rapidly coagulating blood, it is obviously impossible to make such counts. In blood collected with especial precautions and in the slow coagulating blood of hemophiliacs, a general idea of the rates of agglutination and disintegration may be gained by this method. Methods that give an idea of the rate of disintegration of platelets in certain solutions appear preferable to those that attempt to measure their "fragility." The resistance of platelets to disintegration ought, perhaps, to be tested not against solutions of varying osmotic concentration but against substances to the action of which they are ordinarily highly sensitive, for example, tissue juices or the products of cellular disintegration.

1. Jurgens-Naumann Method: Collect venous blood in a paraffin-coated syringe and allow the erythrocytes to settle. Place the plasma in a hematocrit tube (immersed in water at 1 C.) and make counts of the free and of the agglutinated platelets in a hanging drop of the plasma at from one to five minute intervals. In blood from normal persons 90 per cent of the platelets are agglutinated in the first twenty minutes, and 60 per cent, within the first three minutes. In marked thrombopenia the method is impracticable. In two cases of thrombasthenia there was no agglutination until the plasma clotted (Jurgens and Naumann).

2. Modified Howell-Cekada Method: An approximate idea of the rates of platelet agglutination and disintegration may be obtained as follows:

By rapid venous puncture collect 5 cc. of blood into a petrolatum-coated syringe and needle and place the blood in a paraffin-coated tube (between 12 and 15 mm. in internal diameter) at 37 C. Normal blood will remain unclotted for a period of time varying between twenty and fifty minutes. Make platelet counts directly from the specimen at periods of from four to five minutes, handling the blood very carefully and avoiding all shaking. Count the platelets that stand singly on the counting chamber separately from those in unlysed clumps. Plot the results. The first diminution in the number of platelets is due to their agglutination into small clumps; the marked drop that takes place just before coagulation is due to widespread disintegration of platelets. Normally, after thirty minutes very few single platelets can be found.

3. Irish Method: Irish places fixed amounts of suspension of washed platelets in solutions of sodium chloride of various concentrations in a series of tubes. A solution of fibrinogen or of platelet-free plasma is then added. The "fragility" of the platelets is expressed as the concentration of sodium chloride just sufficiently hypotonic to cause disintegration of enough platelets to facilitate rapid clotting.

The method has not had a wide trial, even by its originator. Washing of platelets involves trauma enough to destroy a good portion of the collected platelets, so that the fluid in which they are suspended is always rich in substances active in accelerating clotting, thus vitiating the results.

4. Birch Method: By this method a drop of platelet-rich plasma is suspended in each of various dilutions of salt solution, and swelling of the platelet is used as the criterion of beginning lysis. Birch found that normal platelets lysed at a sodium chloride concentration of about 0.38 per cent. Platelets from the blood of hemophiliacs remained normal in contour in salt concentrations ranging from 5 per cent to pure water for as long as twelve hours.

The advantage of this method is that the platelets are handled very little before they are tested. It is, however, difficult to determine by such criteria as increase in volume the exact point when normal platelets begin to disintegrate. I have seen platelets from the blood of hemophiliacs remain intact for eight hours in a solution of 0.2 per cent sodium chloride.

5. Baar-Szekely Methods of Determining the Rate of Disintegration: First method. Prepare a solution containing: sodium citrate, 2.5 Gm.; mercuric bichloride, 0.05 Gm.; brilliant cresyl blue, 0.5 Gm., and distilled water, 500 cc. Before using this solution add 50 Gm. of urea. Aspirate the solution to the 0.6 mark of a pipet for the counting of leukocytes, then the blood from an incision in the skin up to 1 and the solution to 11. At intervals of twenty, forty, sixty and a hundred and twenty minutes after collection of the blood, count the intact platelets left in the solution in a standard counting chamber and plot the results. In normal blood, from 50 to 75 per cent of the platelets are destroyed in the first forty minutes; there is little change afterward (Baar and Szekely).

Second method (slightly modified). Withdraw *venous* blood into one twentieth of its volume of 10 per cent sodium citrate solution. Use solution-wetted syringes and needles. Place the blood in a paraffin-coated tube and centrifugate it for from one to two minutes at 1,500 revolutions per minute. At twenty, forty, sixty and a hundred and twenty minutes after collection of the blood, count the intact platelets in the plasma, using Tyrode solution as a diluent. In normal blood, by this method, about 20 per cent of the platelets are destroyed in the first forty minutes (Baar and Szekely).

6. Method of Horwitz (1930 [*a*]): Mix a drop of cutaneous blood with about 50 drops of Tyrode solution in a small paraffin-coated tube. Place a drop of the mixture in a counting chamber and establish at once the ratio between the erythrocytes and the platelets. Calculate the absolute number of platelets from the erythrocyte count. At fifteen minute intervals for ninety minutes remove a drop from the well mixed blood dilution and establish the platelet-erythrocyte ratio. Calculate the absolute platelet count from the erythrocyte count and plot the results. In normal blood, by this method, there is a 10 to 15 per cent decrease in the first fifteen minutes and a 15 to 20 per cent decrease in the first thirty minutes; there is little change in the remaining period (Horwitz, 1930 [*a*]).

Adhesion and Clumping of Platelets About Micro-Organisms.—Govaerts Technic: A staphylococcus or Bacillus paratyphosus B is a suitable organism. Withdraw from a normal rabbit by cardiac puncture 20 cc. of blood into a syringe

containing a tenth of that volume of either 1 per cent sodium oxalate or 4 per cent sodium citrate solution. Separate the platelets by the technic of fractional centrifugation (see p. 870) and suspend them in 2 cc. of physiologic solution of sodium chloride after having washed them twice in physiologic solution of sodium chloride. Thus one obtains an emulsion of platelets in strong concentration and free from most of the plasma. Remove also from the same rabbit 10 cc. of blood, allow it to clot and collect the serum. The serum may be used fresh or heated, according to the experiment. Make a salt solution emulsion of the scrapings of a twenty-four hour agar culture of the selected micro-organism. Take 0.1 cc. of the bacterial emulsion, 0.1 cc. of the platelet emulsion and 0.2 cc. of the serum (or plasma, physiologic solution of sodium chloride or heated serum), place them in a small test tube and shake gently for a few minutes. Flocculation and precipitation with clearing of the supernatant fluid indicate agglutination of the platelets and bacteria. When there is agglutination between the platelets alone, the supernatant fluid remains turbid. If platelets and bacteria remain intact and isolated from each other, the mixture appears uniformly turbid, without any evidence of shreds or clumps. Verify the results by direct microscopic examination or from the stained smear.

While simple platelet loading may be observed in the blood of non-immunized animals, the "thrombocytobarine" reaction appears linked with a state of acquired immunity (Brussin and Kalajev).

"Thrombocytobarine" Reaction (Rickenberg's Reaction).—Technic of Brussin and Kalajev: Take 3 parts of physiologic solution of sodium chloride with 2 per cent citrate, 2 parts of blood from the immunized animal and 1 part of the blood containing the corresponding organisms; mix them in a small test tube and place in an incubator at 38 C. for fifteen minutes. Examine a drop of the mixture with and without a stain and look for the characteristic groupings of platelets and bacteria. The phenomenon may be better observed if the citrate-blood mixture is allowed to stand in the incubator undisturbed and the supernatant fluid examined after the erythrocytes have sedimented. For other methods, consult Brussin and Kalajev.

Methods of Studying Other Properties.—For methods of studying the coagulation of blood and the acceleration of clotting through the action of platelets, consult: Morawitz; Fonio (1932); Ferguson (1934 [*b*]); Armitage, Pickering and Mathur, and Eagle.

For methods of studying the induction of syneresis, consult: Le Sourd and Pagniez (1907, 1909 and 1911 [*a*]); Pickering, Mochkovski, and Tocantins (1934 [*a*] and 1936 [*b, d*]).

For methods of studying the bleeding time of the skin, consult: Roskam; Ivy, Shapiro and Melnick, and Tocantins (1936 [*e*]).

For methods of determining the metabolism of platelets, consult Fleischmann, and for methods of investigating their electrical properties, Abramson and Endres (1930).

Megakaryocytes may be cultivated in vitro by the method of Torrioli and Puddu.

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News and Notes

University News, Promotions, Resignations, Appointments, Deaths, etc.—William S. Tillett, associate professor of medicine in Johns Hopkins University, has been appointed professor of bacteriology in New York University.

In the Medical College of Virginia, Paul Kimmelstiel has been promoted to the position of associate professor of pathology and George Z. Williams to that of assistant professor.

Elias P. Lyon, professor of physiology in the University of Minnesota and until recently the dean of its medical school, died suddenly on May 4, in his seventieth year.

Thomas M. Rivers has been chosen to succeed Rufus Cole as director of the Hospital of the Rockefeller Institute for Medical Research.

Linda B. Lange, associate professor of bacteriology in the School of Hygiene and Public Health of Johns Hopkins University, has been appointed professor of bacteriology and immunology in the Woman's Medical College of Pennsylvania.

Lilly Award.—The Society of American Bacteriologists, in conjunction with the American Society for Experimental Pathology and the American Association of Immunologists, is entrusted with the presentation of the Eli Lilly & Company Research Award in bacteriology and immunology. The award consists of a bronze medal and a prize of $1,000 and is given for outstanding research in the fields indicated to a young man or woman not yet 31 years old on April 30 of the year of the award, working in a college or a university. Nominations should be in the hands of the secretary (I. L. Baldwin, University of Wisconsin, Madison) before Sept. 15, 1937.

Abstracts from Current Literature

To Save Space the Original Titles of Abstracted Articles Sometimes Are Shortened

Experimental Pathology and Pathologic Physiology

Effect of Experimental Temporary Vascular Occlusion on the Spinal Cord. L. L. Tureen, Arch. Neurol. & Psychiat. **35**:789, 1936.

Through temporary compression of the aorta, Tureen produced in cats ischemia of the lumbosacral region of the spinal cord. The compression was followed by intravenous injection of 2 cc. of a 4 per cent aqueous solution of neutral red. The compression of the aorta lasted for from fifteen to ninety minutes, and the animals were allowed to live for from twenty minutes to seventeen weeks. The compression resulted in paralysis of the posterior extremities, but the animals that had been subjected to ischemia of fifteen minutes' duration were able to walk within twenty-four hours after the operation. The changes in the spinal cord varied according to the duration of the ischemia. Thus changes in the ganglion cells of the anterior horns were not noted within fifteen minutes after the ischemia but were quite definite within the first hour. The usual changes were swelling of cell bodies and chromatolysis of the Nissl substance. Interstitial changes set in at from forty-eight to seventy-two hours, while satellitosis and neuronophagia were rare. Chromatolysis was complete in three days, after which "reaggregation" of the Nissl bodies into irregular patchy clumps began. Curious facts were noted — occurrence of the severest changes in the ganglion cells between seven and seventy hours after the disappearance of the paralysis and paraplegia in the absence of detectable histologic changes. G. B. Hassin.

Experimental Catalepsy in Cats. W. R. Ingram, R. W. Barris and S. W. Ranson, Arch. Neurol. & Psychiat. **35**:1175, 1936.

By means of the Horsley-Clarke stereotaxic instrument lesions were produced at the base of the cat brain, from the posterior hypothalamus to the oculomotor nerves, along the sides of the cerebral aqueduct and third ventricle. The cerebral aqueduct itself and the substantia nigra were not involved. There was prompt onset of lethargy and somnolence which, however, were not permanent. The animals assumed odd attitudes (catalepsies), showed no interest in food, required tube feeding and lacked all emotional reactions. In the initial stage there was increased tonus of the extensor muscles, and in some instances there was also hypertonicity of the muscles, especially the flexor muscles of the neck and trunk, including those of the abdominal walls. In eleven cataleptic cats the calcium content was found slightly decreased, but the authors do not claim that hypocalcemia is a necessary constituent of the cataleptic syndrome in cats. They found similarities between feline catalepsy and that produced by bulbocapnine. Another interesting observation was that lesions of the central gray matter of the caudad portion of the third ventricle and that surrounding the rostral part of the sylvian aqueduct usually observed in lethargic encephalitis and held responsible for the somnolence did not produce cataleptic phenomena. G. B. Hassin.

Regeneration of Posterior Root Fibers in the Cat. H. A. Paskind, Arch. Neurol. & Psychiat. **36**:1077, 1936.

Paskind tried to determine in cats whether posterior root fibers regenerate. He cut the roots, some in the cervical and some in the dorsal region, and kept the animals alive for approximately from one to twelve months. He gives a detailed description of the changes in the cut roots in a cat which survived 374

days. No signs of regeneration of the intraspinal portions of the root fibers were observed, but the extraspinal portions, distal to the pia-glial membrane, exhibited regeneration, mainly as Büngner's bands. Nerve fibers were occasionally seen also in the intraspinal portions of the posterior roots. Paskind thinks that they probably landed there with the blood vessels or represent dendrites of a ganglion cell of Clarke's column or were efferent fibers of the posterior roots, originating in the cord. This, however, is not considered by him sustainable.

G. B. HASSIN.

THE ADRENAL CORTEX. G. A. HARROP, W. M. NICHOLSON and M. B. STRAUSS, J. Exper. Med. **64**:233, 1936.

The withdrawal of maintenance injections of the adrenal cortical hormone from adrenalectomized dogs during balance experiments in which a constant meat diet was given with constant intake of fluid and salt was followed by increased urinary loss of sodium and chloride and by retention of potassium and nitrogen. When the water intake was low, definite diuresis usually accompanied this excretion of sodium and chloride, but when fluids were forced, no diuresis was observed. The reinjection of the cortical hormone in adrenal insufficiency caused active renal excretion of potassium; the amount excreted was greatly in excess of the probable extra accumulation of this component in the extracellular fluids during the period when insufficiency was developing. This excretion of potassium is surmised to be sufficient to account for such accumulation if diffusible potassium is present in like concentration equally throughout all of the body water, intracellular as well as extracellular. The excretion of potassium was accompanied by a corresponding excretion of phosphate and nitrogen. The excretion of electrolytes which was associated with withdrawal and with subsequent reinjection of adrenal cortical hormone differed from that produced with various diuretic agents regarding which data are available. The effects produced by injection of the cortical hormone during adrenal insufficiency did, however, resemble those produced with posterior pituitary, particularly in the greatly increased excretion of potassium relative to sodium and in the coincidental dilution of the circulating blood. They suggest the possibility that the two similar effects may be ascribable to a common cause.

FROM THE AUTHORS' SUMMARY.

CHANGES IN OUTLYING BONE MARROW ACCOMPANYING A LOCAL INCREASE OF TEMPERATURE WITHIN PHYSIOLOGIC LIMITS. C. HUGGINS and B. H. BLOCKSOM JR., J. Exper. Med. **64**:253, 1936.

In adult mammals and birds a great difference exists in the marrow of the central bones as compared with that of the outlying bones, the distal bones being at a great disadvantage from the standpoint of the production of blood cells. Several experimental procedures are reported by which this disadvantage is overcome, and in consequence the fatty marrow of the outlying bones is replaced by red marrow; this replacement occurs chiefly at the epiphysial regions unless the stimulus of low oxygen is also provided; then the marrow of the diaphysis becomes involved. A common factor in all of the experiments was an elevation of temperature beyond that prevailing in these distal regions, and it is felt that the evidence warrants the opinion that the cause of the improvement is thermal. In some experiments, the formation of blood cells was increasing while the heat was affecting the testis adversely. The experiments permit construction of a general theory of the distribution of fat in the marrow. In certain grafts of precartilage to other rats, normal differentiation into bone, cartilage and marrow occurred, while in others cartilage and very small amounts of primitive marrow developed with slight or no formation of bone. Cartilage was always successfully ingrafted. The capacity to form sinusoids in marrow is determined by the nature of the tissue rather than by the ingrowing endothelium.

FROM THE AUTHORS' SUMMARY.

AN INCREASE IN RETICULO-ENDOTHELIAL CELLS IN OUTLYING BONE MARROW CONSEQUENT ON A LOCAL INCREASE IN TEMPERATURE. C. HUGGINS and W. J. NOONAN, J. Exper. Med. **64**:275, 1936.

In adult mammals and birds there is a great quantitative difference in the reticulo-endothelial content of the marrow of the central bones as compared with the distal outlying bones. Experimental procedures effecting development and increase of hemopoiesis in inactive yellow marrow (see foregoing abstract) also effect an increase in the reticulo-endothelial cells.

FROM THE AUTHORS' SUMMARY.

MUCIN AS AN AID IN THE EXPERIMENTAL PRODUCTION OF LOBAR PNEUMONIA. W. J. NUNGESTER and L. F. JOURDONAIS, J. Infect. Dis. **59**:258, 1936.

Small amounts of sterilized gastric mucin injected intrabronchially aided greatly in the production of lobar pneumonia in white rats. Very small doses of pneumococci, as few as a hundred bacteria, when inoculated with 0.1 cc. of mucin produced lobar pneumonia in about 50 per cent of the animals inoculated. By increasing the number of organisms injected from ten to a hundred times, the incidence of pneumonia was increased to 84 per cent in a series of eighty-six animals. When similar doses of organisms were inoculated in physiologic solution of sodium chloride, the incidence of pneumonia was strikingly lower. In the course of pneumonia in rats the temperature drops below normal after an initial rise. The different stages of lobar pneumonia in the rat are similar to those in the human disease.

FROM THE AUTHORS' SUMMARY.

EXPERIMENTAL CONTRIBUTIONS TO THE STUDY OF HYDRONEPHROSIS. E. C. CRACIUN and D. ZANNE, Ann. d'anat. path. **12**:643, 1935.

Craciun and Zanne performed unilateral ligation of the ureter in thirty-six rabbits and seven dogs, at the same time filling the renal pelvis with a colored material. Gross and microscopic as well as roentgenographic studies were made. They found that in the early stages the kidney increases in size not simply because of dilatation of the renal pelvis but because of extreme interstitial edema. Following this, secretory function may persist if some form of compensation becomes established and resorption from the pelvis takes place; in this event hydronephrosis develops. If resorption does not take place, secretion stops and atrophy of the kidney occurs. They found that resorption from the pelvis takes place by way of the veins and lymphatics, through the interstitial tissue and beneath the capsule. The most important channel for reflux was the vein. PERRY J. MELNICK.

LOCALIZATION OF THE HYPERERGIC INFLAMMATORY REACTION. R. KNEPPER, Virchows Arch. f. path. Anat. **296**:364, 1935.

The hyperergic inflammatory reaction is characterized by fibrinoid degeneration and necrosis of blood vessels and connective tissue. The reaction is not only morphologically characteristic, but it is specific in that it is the result of the interaction of antigen and antibody in the allergic organism. In the localization of the reaction, however, nonspecific factors may play a part and may help to bring about those clinical and pathologic differences that occur in the various diseases that are presumed to have an allergic background. Theoretically, such nonspecific factors might be local stasis, hyperemia, functional activity, hormonal action or increased capillary permeability. The experiments reported were designed to test the effect of certain nonspecific factors in bringing about localization of the hyperergic reaction in the allergic animal. Rabbits were sensitized to swine or horse serum. The homologous serum, injected intravenously or intraperitoneally, served as the specific mediating factor. In one series heat or cold was applied to the ear of the animal. In another series the animals were exercised in a treadmill. In still another series the animals received repeated injections of a suspension of

kidney tissue for its supposed cytotoxic effect on the kidney, and in a final series the pressor principle of the posterior lobe of the hypophysis was injected. Many of the animals died, and many yielded negative results. From the histologic study of his material Knepper gained enough data to convince him of the correctness of his thesis that nonspecific agencies may be a factor in the localization of the hyperergic reaction.

O. T. SCHULTZ.

EXPERIMENTAL HISTAMINE INTOXICATION AND INFLAMMATION. H. HEINLEIN, Virchows Arch. f. path. Anat. **296**:448, 1935.

Rabbits received daily injections of from 0.1 to 0.2 mg. per kilogram of body weight of a solution of histamine (0.4 mg. per cubic centimeter). The duration of administration varied from four to forty-seven days. The organs were examined histologically. In addition to degenerative changes in the parenchyma, more marked reactions, of an inflammatory character, were observed in the blood vessels and connective tissue. These consisted in: desquamation of capillary endothelium; localized swelling of endothelium, sometimes associated with proliferation; escape of plasma into the pericapillary connective tissue; thrombosis of blood vessels; perivascular granulomatous lesions, and organization of the escaped plasma, with fibrosis. These changes were most marked in the lung, heart and liver. The tissue reaction is similar to that of hyperergic inflammation. Heinlein therefore concludes that the hyperergic inflammatory reaction may be the result, in part, of histamine action.

O. T. SCHULTZ.

Pathologic Anatomy

COARCTATION OF THE AORTA WITH INTERMITTENT LEAKAGE OF A CONGENITAL CEREBRAL ANEURYSM. T. W. BAKER and W. D. SHELDEN, Am. J. M. Sc. **191**:626, 1936.

Cardiovascular anomalies are frequently associated with coarctation of the aorta. These anomalies include: a bicuspid aortic valve, defects of the aortic septum, subaortic stenosis, a persistent left superior vena cava, an anomalous origin of the arteries from the aortic arch, and a congenital cerebral aneurysm. Of these associated variations, only the cerebral aneurysm presents a source of potential danger. About 6 per cent of the cerebral aneurysms occurring in young persons have been proved to be syphilitic. The remainder are congenital. When cerebral aneurysms are of syphilitic origin they usually are found in the vertebral or basilar arteries, often assume large size, and give symptoms similar to brain tumor. Congenital cerebral aneurysms, on the other hand, tend to be of small size, are usually present in the smaller cerebral vessels or the circle of Willis, and usually give rise to symptoms only when intermittent leakage or rupture occurs. The average duration of life is about thirty-five years. A case is reported in which coarctation of the aorta with associated intermittent leaking of a congenital cerebral aneurysm was diagnosed before death.

STUART A. SCHLOSS.

THE CHANGES IN THE APPEARANCE OF THE WALL OF A MUSCULAR ARTERY BETWEEN DIASTOLIC AND SYSTOLIC BLOOD PRESSURES. R. J. M. GALLOWAY, Am. J. Path. **12**:333, 1936.

The number of folds in the intima and internal elastic lamina of muscular arteries is a criterion of the degree of contraction of the muscle tissue in the media. The degree of postmortem contraction in excised arteries is not uniform. It varies not only in different arteries from the same person but in different parts of the same segment of an artery. These differences are due largely to differences in stimuli produced in excising the arteries, although other factors also probably play some part. Relaxed excised arteries, when distended by pressures equivalent to at least 80 mm. of mercury or more, show a loss of waviness of the intima

and of the elastic tissues in the wall. The elastic fibers in the media and in the external elastic lamina lose their wavy contour before the fibers in the internal elastic lamina do. The changes in the arterial wall during the passing of the pulse wave probably vary between moderate folding of the intima and internal elastic lamina at diastolic blood pressure and lesser folding at systolic pressure, a change that may even extend to a complete loss of folds in these layers.

FROM THE AUTHOR'S SUMMARY.

THE VISCERAL LESIONS IN SCARLET FEVER AND RELATED STREPTOCOCCUS INFECTIONS. H. BRODY and L. W. SMITH, Am. J. Path. **12**:373, 1936.

The underlying lesion is a vascular injury with concurrent perivascular round cell infiltration. These changes were noted in from 75 to 95 per cent of the hearts, livers, kidneys, adrenals and spleens studied and to a variable degree in the other viscera, including the pituitary gland, lung and pancreas and even the testis. Evidence suggests that these lesions are results of the circulation of a toxin. This is borne out by a certain amount of experimental evidence, which is reviewed briefly.

FROM THE AUTHORS' SUMMARY.

REACTING CELLS IN THE BRAIN IN THE PRESENCE OF A FOREIGN BODY. G. B. HASSIN, Arch. Neurol. & Psychiat. **36**:231, 1936.

A pledget of cotton inserted into the brain of each of a number of dogs by way of the fourth ventricle caused reactive cell changes. These changes varied according to the length of time the foreign body remained in the cerebral tissues, the extent of the injury to the parenchyma and the type of the tissue involved. Thus, in the area occupied by the pledget the ganglion cells exhibited profound degenerative changes and were in a state of marked neuronophagia. The cotton fibers were invaded by lymphocytes and plasma cells (especially in the early stages), fibroblasts and foreign body giant cells. In advanced stages of several weeks' duration, a part of the area occupied by the foreign body was transformed into a connective tissue scar. The zone around the foreign body (enveloping ring) also contained fibroblasts and lymphocytes, but these were mixed with histiocytes, myelophages and other precursors of gitter cells (fat granule bodies) but only in specimens from dogs that died from three and a half to six months after operation. Macrophages were especially numerous in the next zone bordering on the apparently normal brain tissue. Here there were in evidence histiocytes mixed with microglial cells, which were also promiscuous throughout the brain tissues outside the foreign body. There was no evidence of phagocytic activity on the part of these cells, which, like the glia, may become transformed into gitter cells. In the present observations the formation of gitter cells could be traced not to the microglia but to the glia, which exhibited the evolutional stages between oligodendroglia and typical fat granule bodies. FROM THE AUTHOR'S ABSTRACT.

CORTICAL ENCEPHALOMALACIA IN INFANCY. P. M. LEVIN, Arch. Neurol. & Psychiat. **36**:264, 1936.

In a child born by normal delivery, hemiplegia developed at the age of 9 months, following convulsions, which began at the age of 4 months and were followed by unconsciousness and occasional moderate elevation of the temperature. Operation revealed a large cavity in the left superior parietal region. At necropsy a hyperemic cortex was found, at some places softened and slight yellow, and the basilar artery was thick. Microscopic observations revealed decrease in the number of ganglion cells in the third cortical layer; the remaining cells were "disrupted"; numerous small vascular areas of softening were scattered in all lobes of both hemispheres, especially in the parietal regions. In these foci marked vascular changes were present—the capillaries were proliferated, and the endothelial cells appeared hypertrophied or flattened and were mixed with or surrounded by

"adventitial cells" of Marchand. Many phagocytic cells contained fat, though only a very small number of perivascular cells appeared vacuolated. Occasionally the cells were calcified. The "intervascular" cell changes were in the form of ameboid and "globoid" cells; astrocytes or fibroblasts were hardly present. The basal ganglions and the pons were practically normal, but the blood vessels of the pia exhibited marked changes—hyaline-like degeneration, calcification of the media and narrowing of the lumen but no vascular occlusion; the intima in the middle cerebral artery was hyperplastic, and the elastica was split in some places. Vascular disturbances are considered by Levin the probable cause of the cortical changes, which gave a clinical picture of so-called infantile cerebral paralysis.

G. B. HASSIN.

DUPLICATION OF CONGENITAL MALFORMATIONS IN BROTHERS AND SISTERS AND AMONG OTHER RELATIVES. D. P. MURPHY, Surg., Gynec. & Obst. **63**:443, 1936.

Data are presented concerning the frequency with which congenital malformations are duplicated in a series of forty consecutive families, supplemented by observations dealing with the duplication of congenital malformations among siblings in nineteen nonconsecutive families and the duplication of congenital abnormalities among other relatives of a consecutive series of families. In the first group it was found that the defect observed in the first malformed child reappeared in a subsequent malformed sibling in about 50 per cent of all cases. In the second group, in which the malformed child had a malformed relative, the malformation in the child and that in the relative were identical in approximately 41 per cent of the cases. In nineteen nonconsecutive families with two or more malformed children, the defect of the first child was repeated in a subsequent child in over half of the families. Analysis of these facts leads to the conclusion that many if not most of the congenital malformations encountered in this study were the result of defects in the germ plasm which were present prior to fertilization. Further, the frequency of repetition of congenital malformations may be of sufficient importance to cause the obstetrician to suspect the possibility of congenital abnormality and even to aid him in recognizing the type of defect present.

WARREN C. HUNTER.

THE EXCRETION OF URINARY PORPHYRIN IN RHEUMATIC FEVER. E. M. KAPP and A. F. COBURN, Brit. J. Exper. Path. **17**:255, 1936.

The urine of patients with rheumatic fever contains abnormally large amounts of coproporphyrin, qualitatively indistinguishable by visual spectrometric methods from the porphyrin normally present in the urine of healthy persons. Coproporphyrin isolated from the urine of rheumatic patients has been provisionally identified as isomer III. The urine of rheumatic patients nearly always contains part of the coproporphyrin in combination, presumably with a metal. This combined porphyrin has been observed only sporadically in urine from other sources. The increase of urinary porphyrin appears concurrently with the symptoms of rheumatism. There is no increase following recovery from hemolytic streptococcic throat infections in the absence of a rheumatic attack. The porphyrinuria of acute rhuematism is therefore related to the rheumatic process and not to the preceding respiratory infection.

FROM THE AUTHORS' SUMMARY.

LEUKO-ERYTHROBLASTIC ANEMIA. J. M. VAUGHAN, J. Path. & Bact. **42**:541, 1936.

Leuko-erythroblastic anemia is characterized by an abnormal number of extremely immature red cells and a few young myeloid cells in the peripheral blood. The anemia is not necessarily severe; exceptionally it may be megalocytic and hyperchromic Leuko-erythroblastic anemia may occur in various conditions associated with bone change. It has not been seen during the present investigation unassociated with bone changes, though similar disorders of bone were found

in patients with no anemia. Leuko-erythroblastic anemia has not invariably been associated with any peculiarity of calcium metabolism. It occurred with a normal, a diminished and an increased output of calcium. The majority of the patients showed increased excretion of calcium. The incidence of leuko-erythroblastic anemia in carcinomatosis does not appear to be influenced by the nature of the primary growth, by the length of the history or by the character of the bony change. The incidence of leuko-erythroblastic anemia is not dependent on mechanical blocking of the marrow, since it is invariably associated with an increase rather than a decrease of skeletal red marrow, while equal or greater degrees of occlusion of the marrow may be associated with no such anemia. The histologic alteration in erythropoiesis has features akin to those found in addisonian anemia, which suggests that it is caused by deficiency of a factor or factors necessary for normal hematopoiesis.

FROM THE AUTHOR'S SUMMARY.

THE FATE OF IODIZED POPPY-SEED OIL FORTY PER CENT IN THE PULMONARY PARENCHYMA IN MAN. F. BEZANÇON, J. DELARUE and M. VALET-BELLOT, Ann. d'anat. path. **12**:229, 1935.

On the basis of an anatomic study of three cases in human beings and experiments on animals, the authors conclude that iodized oil injected into the bronchial tree reaches the pulmonary alveoli and that the greater part of it is very soon expectorated, leaving only a minimal amount in the air sacs. This very small residue is removed from a healthy lung like any other foreign material, i. e., by mononuclear phagocytes, with practically no inflammatory reaction. The oil becomes dissociated and is carried away. They explain the abnormal persistence of the oil for a long period of time in some cases as follows: If iodized oil is injected into a diseased or altered lung which has not this dissociating power, it will become deposited in the interstitial tissue and remain there.

PERRY J. MELNICK.

PRIMARY LYMPHOGRANULOMATOSIS OF THE ESOPHAGUS. J. CHIOLERO, Ann. d'anat. path. **12**:305, 1935.

The clinical and autopsy record of a case of Hodgkin's disease of the esophagus is presented. Only the esophagus was involved; neither the lymph nodes nor any other organ contained Hodgkin's granuloma. Only three other cases of Hodgkin's disease of the esophagus are recorded in the literature; they differed from this case in that lymph nodes were also involved.

PERRY J. MELNICK.

MASSIVE ACUTE ATROPHY OF THE LIVER. A. SCHRUMPF, Ann. d'anat. path. **12**:447, 1935.

An acute yellow atrophy of the liver developing in a 31 year old woman two months after recovery from puerperal sepsis was so extensive that Schrumpf feels justified in naming it "massive acute atrophy" of the liver. There was almost total hepatic insufficiency, and, as in experimental hepatectomy, there were marked hypoglycemia, decrease in the blood urea and increase in blood amino-acids.

PERRY J. MELNICK.

KARYOCLASTIC POISONS. M. ICKOWICZ, Ann. d'anat. path. **12**:501, 1935.

Ickowicz injected salts of arsenic, nickel and cobalt into rats and studied their effect on the nuclei, especially those of the thymus gland. He found that small doses produce an increase in mitoses and that the dose that produced extensive pyknosis of the nuclei was very close to the toxic dose. In this the salts used resemble many other substances, depending on the dose. The author sees no justification for classifying certain chemicals as a separate group of karyoclastic drugs.

PERRY J. MELNICK.

VARICOSIS AT THE BASE OF THE BRAIN. J. ROMANOWSKI, Centralbl. f. allg. Path. u. path. Anat. **64**:210, 1936.

Varicosities in the left middle and left basal cerebral veins were found in the body of a woman 56 years old who died of lobar pneumonia. The dilatations appeared as nodular convexities and were from 5 to 16 mm. in diameter. Microscopic study of the walls of the varices disclosed numerous foldings and hyaline degeneration. The thickness of these walls was from 0.1 to 2 mm. Most of the dilated veins were occluded with antemortem thrombi. The clinical data and the necropsy offered no explanation for the production of the varices. Ten records of varicosis in cerebral veins appear in medical literature; in seven of the cases tearing of a varix resulted in fatal hemorrhage. Anatomic changes of the wall of the involved vein following infectious processes and increased intravenous pressure are important factors in the formation of varices.

LOUISA HEMKEN.

LIPOID CELL GRANULOMA OF THE FALLOPIAN TUBES AFTER UTEROSALPINGOGRAPHY. G. NEUMEYER, Centralbl. f. allg. Path. u. path. Anat. **64**:241, 1936.

A 34 year old woman had severe abdominal pain several months after an injection of 5 cc. of iodized poppy-seed oil 40 per cent for uterosalpingography. The tubes and uterus were removed. The uterus was normal; both tubes were distended, and the walls measured from 1.5 to 3 cm. in diameter. There were numerous miliary cystlike spaces in the yellow mucosa, and the dilated lumen was filled with crumbly gray-yellow material. There were chronic inflammatory thickening of the wall and focal collections of pale macrophages beneath the epithelium. The contents of the macrophages and the material in the lumen appeared red with sudan III and blue with the nile blue sulfate stain. These conditions indicate that iodized oil at times may cause serious changes in the fallopian tubes.

LOUISA HEMKEN.

ESOPHAGEAL DIVERTICULA. L. E. C. SCHMIDT, Centralbl. f. allg. Path. u. path. Anat. **65**:33, 1936.

Traction diverticula of the esophagus were present in a 7 year old boy dying from diphtheria. Two small diverticula were located in the anterior wall of the upper third of the esophagus; a larger one was present near the level of the bifurcation of the trachea. Furthermore, there were two islands (each about 2 by 0.5 cm.) of gastric mucosa in the esophagus, a short distance below the larynx. Inflammatory changes could not be detected in the walls of the diverticula and adjacent tissues. The occurrence of traction diverticula associated with aberrant gastric mucosa in the esophagus points to a congenital origin.

LOUISA HEMKEN.

FACTORS INFLUENCING THE DEPOSITION OF PIGMENT IN HEART MUSCLE. H. MÜLLER, Virchows Arch. f. path. Anat. **295**:514, 1935.

The nucleus and the surrounding zone of pigment deposition were measured in various areas of 100 hearts of all age groups. Müller concludes that myocardial pigmentation is a physiologic process that is correlated with work hypertrophy of the heart and is not influenced by pathologic processes. The latter may disturb quantitatively the usual normal distribution of the pigment.

O. T. SCHULTZ.

RELATION BETWEEN DIAMETER OF THE AORTA AND DISTRIBUTION AND EXTENT OF ATHEROSCLEROSIS. E. DORMANNS and E. EMMINGER, Virchows Arch. f. path. Anat. **295**:525, 1935.

In 1,000 necropsies of persons over 20 years old, the width of the aorta was measured at three different levels and this factor correlated with the degree of

atherosclerosis present. The conclusion was reached that the diameter of the aorta in itself is not a factor in atherosclerosis. Localized weakening of the wall, which may lead to irregular dilatation, appears to be a factor in the distribution of atherosclerosis.

O. T. SCHULTZ.

PATHOGENESIS OF TUBERCULOUS MENINGITIS. CHRISTIANNE RADMANN, Virchows Arch. f. path. Anat. **295**:563, 1935.

Ten brains with tuberculous leptomeningitis were examined with the aim of determining the correctness of Rich's view that in the majority of instances tuberculous meningitis is not the result of hematogenous infection of the meninges but results from the spread of an older cerebral or meningeal focus. The observations in three of the ten brains are held to confirm Rich's contention. In each of these there was an older process with terminal miliary tuberculosis; i. e. there were cerebral tubercles older than the terminal miliary lesions. Four brains showed progressive primary tuberculosis with overwhelming terminal involvement. The author believes that meningitis of this type can be distinguished histologically from that secondary to an older lesion of the brain or meninges. The conditions in the remaining three brains are placed in an intermediate group. In one of these there was a solitary tubercle of the brain, but histologically the meningeal process was held to be like that of the second group. In the other two a primary complex could not be found.

O. T. SCHULTZ.

SUPRASCAPULAR BRANCHIAL CYST. H. HAMDI and K. S. UGE, Virchows Arch. f. path. Anat. **295**:576, 1935.

A supraclavicular cyst in a boy aged 13 years, which had been evident since the age of 4 years, was surgically removed. From a fistulous opening there had escaped a clear mucoid liquid. Microscopic examination showed the lining of the cyst to consist, in places, of squamous epithelium; in other places, of ciliated epithelium of the respiratory tract, and, in the greater portion, of gastric mucosa. The cyst is held to have been derived from the first branchial arch at an early embryonic period when the epithelium still had its multiple potencies of forming three different kinds of differentiated epithelium.

O. T. SCHULTZ.

WASTE PIGMENT OF RENAL EPITHELIUM. N. BROCK, Virchows Arch. f. path. Anat. **295**:578, 1935.

The occurrence of waste pigment in the epithelium of the transitional portion of the renal tubules is a normal physiologic process. The degree of pigmentation is influenced by age and by certain pathologic states. In 210 kidneys of all ages pigment was never seen in the capsular epithelium or endothelium of the glomeruli. The absence of pigment in the capsular lining cells of the glomeruli gives no clue as to the origin or nature of these cells. From the second decade on, fat droplets are seen normally in the glomerular endothelium.

O. T. SCHULTZ.

Microbiology and Parasitology

CHRONIC DEFICIENCY OF VITAMIN C IN TUBERCULOSIS IN THE GUINEA-PIG. M. R. GREENE, M. STEINER and B. KRAMER, Am. Rev. Tuberc. **33**:585, 1936.

In guinea-pigs chronic deficiency of vitamin C combined with progressive tuberculous infection causes a significant shortening of the survival period and a significant decrease in body weight. Chronic scurvy did not cause generalization of tuberculosis in animals infected with the relatively avirulent strain RI. Generalized tuberculosis developed more rapidly in animals chronically deficient in vitamin C than in nonscorbutic animals when both were infected by the subcutaneous or enteric route with strain H 37 or tuberculous sputum. Guinea-pigs maintained

on a diet partially depleted of vitamin C acquired more lesions and ulcers in the intestinal tract than did animals on a complete diet when both were fed tubercle bacilli. When infected tuberculous sputum was fed, the extent and severity of the tuberculosis in the intestinal tract were greater than when pure cultures of tubercle bacilli were administered. Animals infected with tubercle bacilli and allowed to become acutely scorbutic showed no more tuberculosis than the control guinea-pigs.

H. J. Corper.

Irradiated Milk in Experimental Inhalation Tuberculosis in Guinea-Pigs. D. F. Loewen and W. H. Oatway Jr., Am. Rev. Tuberc. **33**:733, 1936.

No remarkable prophylactic or therapeutic effects were found in guinea-pigs (normal or sensitized) given irradiated cream and infected by the "spray-inhalation" method. The groups receiving irradiated cream exhibited a sustained higher average weight curve than did the control groups. Calcium deposits were found during gross and microscopic examination only in the tissues (and lesions) of tuberculous animals. The calcium content of the lungs of the infected animals was greater than that of the uninfected, and was in quantitative relationship to the amount of disease present in the lungs. The occurrence of calcification and of an abnormal calcium content of the lungs was independent of the vitamin and of the calcium intake of the animals. The administration of irradiated cream was not followed by untoward effects.

H. J. Corper.

Dissociation of Tubercle Bacilli, with Special Reference to the Avian and Human Types. Eleanor Alexander Jackson, Am. Rev. Tuberc. **33**: 767, 1936.

Three strains of human tubercle bacilli were dissociated. Human S colonies are convex, round, smooth-edged and glistening and resemble bovine and avian S colonies. Small optimal amounts of ferric chloride (0.0004 per cent) added to the Bordet-Gengou medium favor S colonies in pure culture. Lack of sufficient blood in the Bordet-Gengou medium, and of crystal violet in Long's medium, is inhibitory to the formation of S colonies. Crystal violet in 0.01 per cent concentration discouraged avian S and in 0.001 to 0.0001 per cent concentration inhibited human S colonies. The lesions produced by avian and human R bacilli tended to be limited, calcified and macroscopic; they contained relatively few bacilli, while S organisms produced more diffuse small or microscopic inflammatory lesions, containing more bacilli. The S bacilli of a given strain caused more symptoms of illness and emaciation and were somewhat more virulent than the R bacilli of the same strain. In the case of two strains the R bacilli of one strain were more virulent than the S of the other strain. One of thirteen serums from tuberculous patients was able to dissociate completely the human tubercle bacillus (H3 and H37) from R to S in one generation. The serum of a nontuberculous person who showed a positive Mantoux reaction was able to dissociate these human strains completely in one generation. The complement-fixing power of the serum bore no relation to the dissociating power. The serum of two rabbits immunized against a protein fraction of the tubercle bacillus (H37) did not dissociate strains H3 and H37. A catalyst mechanism is suggested as underlying the dissociation phenomenon.

H. J. Corper.

Protective Action of Certain Chemicals Against Infection of Monkeys with Nasally Instilled Poliomyelitic Virus. A. B. Sabin, P. K. Olitsky and H. R. Cox, J. Exper. Med. **63**:877, 1936.

Aluminum sodium sulfate ($Al_2Na_2[SO_4]_4.24H_2O$) or tannic acid instilled intranasally in proper concentration and over a period of several days protects monkeys against subsequent infection by the nasal route with the virus of poliomyelitis.

While mice are protected against the virus of equine encephalomyelitis when a 0.5 per cent solution of either tannic acid or aluminum sodium sulfate is used, the minimal concentration effective in preventing poliomyelitis in monkeys is 3 per cent, indicating different requirements as regards variations either in the host or the virus. Treatment over a period of at least three days is required to induce resistance which, once developed, does not disappear quickly when the nasal instillations of the chemical are repeated daily. Treatment for three days does not in itself confer sufficient resistance to endure for from forty-eight to ninety-six hours, but when treatment is continued for five days or more the majority of monkeys (eleven of fifteen) are still refractory about a month later and about half the number (seven of fifteen) two months later, while at the end of three months normal susceptibility again prevails. Neither tannic acid nor alum acts directly on the virus in a manner to prevent it from causing poliomyelitis by way of the nose; the action of these chemicals is rather on the tissues of the host (olfactory mucosa?); the changes leading to the refractory state require several days to be brought about. Aluminum sodium sulfate does not have an early or primary effect favoring invasiveness of the virus, and the incidence of the disease is either unchanged or perhaps even lower than among monkeys not so treated. Monkeys rendered refractory by treatment fail to exhibit evidence of specific humoral or tissue immunity after exposure to the virus. Four per cent tannic acid or aluminum sodium sulfate can be given to human beings with only slight discomfort and no apparent harmful effects.

FROM THE AUTHORS' SUMMARY.

PHENOMENON OF LOCAL SKIN REACTIVITY TO BACTERIAL FILTRATES. G. SHWARTZMAN, J. Exper. Med. **64**:529, 1936.

Each of a series of rabbits was sensitized by a single intravenous injection of horse serum six days prior to the experiments. In these rabbits there appeared hemorrhagic and necrotic reactions in sites of intradermal tests with horse serum when the tests were preceded, one hour, or followed, eighteen to twenty-four hours later, by intravenous injections of potent bacterial filtrates. The skin-preparatory and reacting potencies of the filtrates were titrated by means of the phenomenon of local skin reactivity to bacterial filtrate. The experiments demonstrated that the ability of bacterial filtrates to elicit reactions in the antigen-treated sites in sensitized animals is strictly conditioned by their provocative potency. Combinations of animal protein antigens with homologous antiserums, which are endowed with provocative potency but are totally devoid of skin-preparatory potency, fail to elicit reactions in antigen-treated sites. The various methods of elicitation of the phenomenon of local tissue reactivity are summarized and discussed in the text.

FROM THE AUTHOR'S SUMMARY.

DISSOCIATION OF UNUSUAL ACID-FAST ORGANISMS. W. STEENKEN JR. and A. LANDAU, J. Infect. Dis. **58**:247, 1936.

We have described three strains of acid-fast organisms, two isolated from human sources and one probably a contaminant. Each strain was dissociated into at least two variants possessing distinct topographic and other biologic characteristics. Some of the variants in the early stages of colony development appeared to be white, but as they aged, when left at room temperature and exposed to light, they invariably showed chromogenicity. All the variants propagated at 37.5 C. as well as at 20 C.; however, at the former temperature their growth was more profuse. Their morphologic and tinctorial characteristics could not be used as a means of differentiation. Nonacid-fast forms could be demonstrated in very young and again in older cultures. They were all very pleomorphic. These studies definitely show that environmental conditions play no small part in morphologic variation. The strains were nonpathogenic for laboratory animals, with the exception of "M," which displayed some pathogenicity for mice and rabbits. It may be that larger doses are required, or it is possible that their virulence will be enhanced

after several passages through the animals. They were weak antigens, producing complement-fixing antibodies with some degree of specificity. Specific tuberculins could be prepared from the strains, and animals inoculated with a strain reacted more strongly to the homologous than to the heterologous tuberculin.

The interpretation of the significance of the various so-called nonpathogenic acid-fast organisms isolated from human sources is still speculative. It seems, however, that one cannot regard them merely as saprophytes, although there are certain similarities, viz., their behavior on artificial mediums, the development of chromogenicity, the p_H curves and their pleomorphic morphology in different environments. It seems highly probable, however, that these organisms may, under certain circumstances, be responsible for the production of disease in man.

FROM THE AUTHORS' SUMMARY.

TYPHUS IN MALAYA. R. LEWTHWAITE and S. R. SAVOOR, Brit. J. Exper. Path. **17**:208 and 214, 1936.

Two strains of the virus of tropical typhus have been isolated from wild rats trapped in endemic areas. Both were isolated and maintained in guinea-pigs. One could be isolated in rabbits by intra-ocular inoculation and maintained in them indefinitely. The features of this infection appeared to be identical with those of corresponding infections obtained with the viruses of rural typhus and human tsutsugamushi disease. Repeated attempts to isolate the other rat strain in rabbits in the same manner failed. Rickettsias were found with ease and in abundance in infected material from guinea-pigs infected with either strain and from rabbits infected with the one strain. The serums of rabbits infected with the two rat strains gave positive Weil-Felix reactions of significant titers. Cross-immunity tests in rabbits between one rat strain and six human strains of rural typhus and tsutsugamushi disease showed a cross-immunity to exist, complete in five strains and partial in the sixth strain. A concomitant infection with sodoku was present in the guinea-pigs of both strains; although this may have modified the clinical signs, the infection by a typhus virus could be determined by four decisive criteria. The conclusion is drawn that the murine origin of the virus of the rural typhus-tsutsugamushi group of diseases is now firmly established.

The Weil-Felix reactions of serums from rabbits and monkeys inoculated with human virus or laboratory virus of rural typhus, urban typhus or the tsutsugamushi disease are reported and discussed, as also are the corresponding reactions of serums from rabbits inoculated with the two strains of the virus of tropical typhus recovered from wild rats. Instances are given of a change in the type of antibody response; in particular, an experience is described in which the agglutinogenic properties of the virus of a strain of tropical typhus underwent a temporary change, while the immunogenic properties remained unchanged.

FROM THE AUTHORS' SUMMARIES.

PROPAGATION OF INFLUENZA VIRUS ON THE DEVELOPING EGG. F. M. BURNET, Brit. J. Exper. Path. **17**:282, 1936.

Virus from a typical case of epidemic influenza has been propagated for more than seventy generations in developing eggs. The lesions produced in the initial passages were trivial, but the pathogenicity of the virus for the embryo has steadily increased. By the seventy-third passage all embryos were killed within forty-eight hours with multiple hemorrhagic lesions in many tissues. Well defined focal lesions, the number of which can be used as an index of the amount of virus inoculated, are produced on the chorio-allantoic membrane by the later generations. The histologic appearance of the lesions of the chorio-allantoic membrane is described and illustrated. The virus has retained its original immunologic characteristics.

FROM THE AUTHOR'S SUMMARY.

THE SEROLOGIC, TOXIGENIC AND BIOCHEMICAL REACTIONS OF HEMOLYTIC STREPTOCOCCI FROM THE THROAT OF HONGKONG CHINESE. L. J. DAVIS and J. S. GUZDAR, J. Path. & Bact. **43**:197, 1936.

The frequency of hemolytic streptococci in the throats of a sample of 788 apparently healthy Hongkong Chinese was found during the winter months of 1936 to be 8.6 per cent. The strains isolated, together with ten additional strains isolated under similar conditions during the previous year, have been classified by the precipitin method of Lancefield. Of the 78 strains examined 28 were assigned serologically to group A, 23 to group C and 27 to group G. During these winter months the carrier rate for nasopharyngeal streptococci belonging to group A was approximately 3 per cent. The toxigenic properties of the strains were studied. With one exception all the cultures of group A yielded toxins capable of producing definite erythematous reactions in a subject whose reaction to the Dick test was positive. None of the strains in group C or G succeeded in this respect, although a few gave rise to small indefinite reactions. The biochemical reactions of the strains have been described. The findings in respect to the cultures of group A accord with those of previous investigators. The behavior of the strains of groups C and G resembled on the whole that of certain types of hemolytic streptococci of animal origin described by other workers.

FROM THE AUTHORS' SUMMARY.

VARIATIONS IN THE PATHOGENICITY OF THE SPIROCHETE OF SYPHILIS. C. LEVADITI, A. VAISMAN, R. SCHOEN and Y. MANIN, Ann. Inst. Pasteur **56**:251, 1936.

A study of the Truffi strain of spirochetes, the activity of which was traced from 1908, indicated marked attenuation so far as pathogenicity for man or anthropoids is concerned. Two laboratory accidents and one inoculation in a human volunteer permitted some conclusions to be drawn regarding the pathogenicity of this strain for man. Local lesions were produced, but there was little apparent tendency to spread. Observations of mice inoculated subcutaneously indicated that infection of lymph nodes is not dependent on presence of the virus in the blood stream. Such inapparent infection of mice was not successfully transmitted in series. The development in situ of a "vegetative" form of the virus was said to account for the presence of the spirochetes in the peripheral lymphatic system. The report is extended to present further studies of the two phases of the syphilitic virus, the invisible aspirochetal and the vegetative spirochetal phase. The latter is believed to develop in the same manner as the former, regardless of the form in the inoculum. These phases were studied microscopically, and an intermediate stage characterized by twisted loops was noted. The resistance to gamma rays, rays from the mercury vapor arc in quartz and glycerin appeared equal.

M. S. MARSHALL.

POSTMORTEM CULTIVATION OF TUBERCLE BACILLI IN RHEUMATIC HEART DISEASE. C. CORONINI and H. POPPER, Virchows Arch. f. path. Anat. **296**:422, 1935.

Löwenstein and later Reitter and Löwenstein reported a striking frequency of positive cultures of the tubercle bacillus from the blood during life in various forms of rheumatic disease. Popper applied the Löwenstein technic to blood obtained post mortem in cases of tuberculosis. Positive results were obtained only in cases of advanced, fatal tuberculosis with fresh miliary tubercles. The present report gives the results of postmortem cultures in a series of cases of rheumatic heart disease. The material consisted of 161 cases investigated in the course of three years by means of 588 organ and 166 blood cultures. The results are recorded as macroscopically positive if visible colonies developed and microscopically positive if acid-fast and alcohol-fast bacilli could be detected in the cultures only microscopically. The organisms cultivated were pathogenic for animals and caused histologically characteristic tuberculous lesions in the animals. The older the rheumatic process the lower was the percentage of positive cultures. The classi-

fication of cases and the results were as follows: 18 cases of acute and subacute heart involvement (pancarditis), 10; 15 cases of recurrent chronic rheumatic endocarditis 7; 45 cases of chronic endocarditis with fresh verrucae, 13; 57 cases of chronic endocarditis, 7. Since many believe that endocarditis lenta develops on the basis of a previous rheumatic injury of a valve, 27 cases of this disease were investigated; 4 yielded positive results. In more than half of all the cases studied there were no gross or microscopic tuberculous lesions. Of the organs cultured, the tonsils and the myocardium gave the largest number of positive results. Accepting the view that rheumatic disease is a hyperergic reaction in an allergic state, the authors interpret their findings as indicating that in certain cases at least rheumatic disease is such a reaction to a second invasion by tubercle bacilli.

O. T. SCHULTZ.

ACID-FAST AND ALCOHOL-FAST BACILLI IN THE URINE OF PATIENTS WITH RHEUMATIC POLYARTHRITIS. F. STENGEL, Virchows Arch. f. path. Anat. **296**: 569, 1936.

The Löwenstein method for the cultivation of tubercle bacilli from the blood stream was used in 176 cases. Of these, 104 were cases of tuberculosis and 58, cases of rheumatic disease; the remaining 14 were controls. Positive cultures were obtained in only 3 cases of tuberculosis and 2 of rheumatic disease. Because the results were so overwhelmingly negative, an attempt was made to detect acid-fast and alcohol-fast bacilli in the urine and to cultivate them. The urine of 121 patients was examined; 93 were adults and 28 were children. Included were 33 patients with rheumatism, 17 with tuberculosis and 71 controls. The sediment of twenty-four hour specimens was centrifugated, stained by the Ziehl-Neelsen method and cultured by the Löwenstein method. From 3 to 95 examinations were made in the individual case in the course of from 3 to 136 days. Acid-fast and alcohol-fast bacilli were found in 51 per cent of the controls, 70 per cent of the tuberculous patients and 84 per cent of the rheumatic patients among the adults and in 50 per cent of the tuberculous children and 38 per cent of the children with rheumatic disease. These differences are not considered significant. Positive results on first examination were obtained for 7 per cent of the controls, 12.4 per cent of the tuberculous and 17 per cent of the rheumatic patients. Tubercle bacilli were cultivated from the urinary sediments in 2 cases of the rheumatic group, 7 of the tuberculous group and 8 of the control group. The relative frequency of acid-fast and alcohol-fast bacilli and the relative infrequency of their cultivation lead Stengel to conclude that the organisms are avirulent variants of the tubercle bacillus.

O. T. SCHULTZ.

ATTENUATION OF TYPHUS VIRUS BY BILE. V. DIMITRIJEVIĆ-SPETH, Zentralbl. f. Bakt. (Abt. 1) **134**:67, 1935.

Typhus virus in brains from infected guinea-pigs was mixed with varying dilutions of ox bile and after varying intervals was injected intraperitoneally into guinea-pigs in tests for evidence of attenuation. Attenuation was obtained. No immunity, however, was secured when such attenuated virus was injected into guinea-pigs; in fact, the vaccinated animals became febrile, showing that the treatment with ox bile did not destroy the virus.

PAUL R. CANNON.

THE VIRUS OF FOOT AND MOUTH DISEASE AS INFLUENCED BY TISSUE CULTURE. E. STRIEGLER and H. C. NAGEL, Zentralbl. f. Bakt. (Abt. 1) **134**:71, 1935.

Seven series of cultures of the virus of foot and mouth disease were given fifty passages through tissue cultures and the virulence, infectivity and immunizing potencies tested. The three types of virus, A, B and C, were used. The virulence was considerably diminished during tissue culture, but the types remained unchanged. Immunization with the culture virus engendered immunity to the original virus.

PAUL R. CANNON.

Immunology

IMMUNOLOGIC STUDIES OF THE VIRUS OF INFECTIOUS LARYNGOTRACHEITIS OF FOWLS, WITH THE "DEVELOPING EGG TECHNIC". F. M. BURNET, J. Exper. Med. **63**:685, 1936.

The technic of inoculation in the chorio-allantoic membrane has been applied to the study of the virus of infectious laryngotracheitis as it occurs in Australian poultry. When suitably diluted suspensions of the virus are inoculated, isolated pocks appear, the macroscopic form and histologic structure of which are characteristic. The number of thsee pocks may be used as a measure of the amount of virus present. Two distinct types are produced by the strains of laryngotracheitis, one being characteristic of epizootic strains from New South Wales and from America, the other of a Victorian strain which is of very low virulence for fowls. No qualitative antigenic differences can be detected among these strains, but the epizootic strains are more readily neutralized by immune serum than the enzootic Victorian strain. A study of the inactivation of the virus by immune serum shows that (*a*) the inactivation requires time for its completion in vitro; (*b*) the proportionate reduction in titer produced by a given concentration of antiserum is independent of the initial concentration of the virus; (*c*) reactivation by dilution is readily demonstrable; (*d*) the virus in the presence of small concentrations of immune serum, producing only slight inactivation, is rendered incapable of passing a graded collodion membrane normally permeable to it; (*e*) the pocks produced with partially neutralized suspensions of virus are smaller, suggesting retardation in attack. These findings bring the neutralization of a typical virus by immune serum completely into line with the phage-antiphage reaction described by Andrewes and Elford.

FROM THE AUTHOR'S SUMMARY.

STUDIES IN SYNERGY. H. F. SWIFT and M. P. SCHULTZ, J. Exper. Med. **63**:703 and 725, 1936.

The degree of immunization and sensitization of rabbits following injections of beef lens is markedly increased when the animals are under the influence of staphylotoxin. Since the effect of the latter is exerted when the two substances are introduced separately into the same tissues, with several hours elapsing between the injections, or into different veins, it appears that an intimate association of the two is unnecessary. A stimulating action of the toxin on the antibody-forming cells is a more probable explanation of the phenomena observed. Animals actively immune to staphylotoxin fail to show any synergic effect of this toxin when it is introduced with beef lens. Neutralization of the toxin in vitro, on the other hand, fails to eliminate this stimulating effect.

The relative synergic stimulating influence of (1) sensitivity to horse serum, (2) hyperergy to the nonhemolytic streptococcus and (3) staphylotoxic intoxication has been determined in connection with rabbits' reactions to simultaneous injections of lens extracts. These three synergic states are increasingly active in the order named. Heterologous lens extract is a much more powerful antigen than is homologous lens extract, even under conditions in which the reactivity of the immunized animal has been much enhanced.

FROM THE AUTHORS' SUMMARIES.

THE IDENTITY OF PRECIPITIN AND AGGLUTININ. M. HEIDELBERGER and E. A. KABAT, J. Exper. Med. **63**:737, 1936.

The absolute quantitative agglutinin method has been used for the determination of the presence or absence of small amounts of specific polysaccharide in variants of the pneumococcus. A technic is described for the removal of group-specific antibody from antipneumococcus horse serum. Not only are the type-specific anticarbohydrate agglutinin and precipitin present in identical amounts in

type I antipneumococcus horse serum, but a reduction in one is accompanied by a quantitatively identical reduction in the other, providing evidence for their identity. In purified antibody solutions somewhat more agglutinin than precipitin is found, possibly owing to alteration of a portion of the antibody in the purification.

FROM THE AUTHORS' SUMMARY.

ACTIVE IMMUNIZATION OF GUINEA-PIGS WITH FORMALDEHYDIZED VIRUS OF EQUINE ENCEPHALOMYELITIS. H. R. COX and P. K. OLITSKY, J. Exper. Med. **63**:745, 1936.

Resistance of a high degree may be induced in guinea-pigs and mice against experimental equine encephalomyelitis by means of vaccines treated with dilute solution of formaldehyde, in which no active virus can be demonstrated. The induced resistance is not due to residual traces of active virus which might possibly have escaped detection in the formaldehydized tissue preparations.

FROM THE AUTHORS' SUMMARY.

GROUP-SPECIFIC A SUBSTANCE IN COMMERCIAL PEPSIN. K. LANDSTEINER and M. W. CHASE, J. Exper. Med. **63**:813, 1936.

A method of preparation and a preliminary chemical investigation of the substance present in commercial (pig) pepsin which reacts with human A antiserum are presented. The material offers an especial advantage in securing in quantity a serologically highly active preparation suitable for further studies. Active preparations were isolated, moreover, from commercial (pig) gastric mucin. Some other materials showing group-specific reactions are mentioned.

FROM THE AUTHORS' SUMMARY.

THE MASS FACTOR IN IMMUNOLOGIC STUDIES ON VIRUSES. M. H. MERRILL, J. Immunol. **30**:169, 1936.

On the basis of an analogy of the total mass of antigen necessary in the precipitation and agglutination reactions with red blood cells, bacteria, crystalline egg albumin and a diazo dye, Merrill calculates the number of antigenic particles, the mass of each particle and the theoretical mass number relationship. From these theoretical considerations he concludes that the reason why immunologic reactions in vitro were so rarely obtained for viruses is not that these are due to a special mechanism but that the concentrations of virus particles employed for such reactions were insufficient. Similar factors are postulated for immunization with viruses and for absorption of immune bodies.

I. DAVIDSOHN.

NEUTRALIZATION OF THE VIRUS OF EQUINE ENCEPHALOMYELITIS BY IMMUNE SERUM. M. H. MERRILL, J. Immunol. **30**:185 and 193, 1936.

1. *Combination of Virus and Antibody in Vitro.*—When the virus of equine encephalomyelitis and a subneutralizing dose of immune serum were mixed and then diluted, the titer by intracerebral inoculation was from ten to a hundred times below the titer of the same materials mixed after dilution. When virus was inactivated by mixing it with immune serum, all dilutions of the mixture were noninfective on intraperitoneal inoculation. When each was diluted before mixing, animals receiving the higher dilutions were not protected. The filtrability of the virus was diminished by mixing with subneutralizing doses of immune serum. It is concluded from these results that the virus and the immune body combined in vitro. It is considered probable that aggregation of the virus particles occurred.

2. *Percentage Law.*—The addition of subneutralizing doses of immune serum to suspensions of the virus of equine encephalomyelitis caused a definite decrease

in the titer of the virus, indicating a 90 to 99 per cent neutralization. This occurred over a wide range of serum-virus ratios. In the narrow zone of serum-virus ratios in which neutralization was complete there was a direct relationship between the number of infective doses of virus present and the amount of serum required for neutralization. It is believed that two phenomena were being measured, namely, aggregation and neutralization, and it is suggested that the phenomenon of percentage neutralization with the virus in question, as well as with phage, is probably due to aggregation of virus, or phage, particles.

FROM THE AUTHOR'S SUMMARIES.

ABNORMAL ISO-ANTIBODIES OBSERVED FOLLOWING TRANSFUSIONS. E. NETER, J. Immunol. **30**:255, 1936.

The serum of a recipient of blood group O with a history of three previous transfusions agglutinated and lysed the blood of a donor of the same group but only when undiluted. The agglutination was stronger at icebox temperature but marked at 37 C. The serum agglutinated five of twenty samples of bloods of group O. The iso-antibody was found again seventeen days later but was absent after twenty-three days.

I. DAVIDSOHN.

HYPERSENSITIVENESS TO POISON IVY IN GUINEA-PIGS. F. A. SIMON, J. Immunol. **30**:275, 1936.

Application of an extract of poison ivy to the surface of the skin brought about hypersensitiveness in guinea-pigs after seven days, but intramuscular, intraperitoneal and intravenous injections did not have the same effect. Exclusion of vitamin C from the diet inhibited the development of hypersensitiveness. The hypersensitiveness was limited to the epidermis. The excision of the treated portion of the skin did not prevent the establishment of hypersensitiveness unless it was done not later than from eighteen to twenty-four hours after the application of the extract. The hypersensitiveness could not be transferred with the serum. No definite evidence was obtained concerning the method of spread of hypersensitiveness from the area of application to the remaining surface of the body, but the results favor the blood stream or the lymphatic system as conveyers in preference to the epidermis. Similarly, it is suggestive that the distributed substance is an altered allergen. Histologic sections of the lesions showed inflammatory infiltrations and necrosis, which appeared earlier and were more marked in the sensitized animals. Attempts to sensitize rats, rabbits and cats were unsuccessful.

I. DAVIDSOHN.

AGGLUTINOGENS M AND N OF THE AINU. T. KUBO, J. Immunol. **30**:287, 1936.

Kubo found the distribution of the properties M and N among 504 Ainu to be: type M, 17.85 per cent; type N, 31.94 per cent; type MN, 50.20 per cent. If s and t represent the frequencies of genes M and N, respectively, the races of the world may be divided into three groups as follows:

(1) $\frac{s}{t} > 2$. The only race in this group is the American Indian.

(2) $2 > \frac{s}{t} > 1$. In this group most of the races of the world fall.

(3) $\frac{s}{t} < 1$. This group includes the Ainu and the Javanese.

A. S. WIENER.

ACTIVE IMMUNIZATION AGAINST TETANUS INFECTION WITH REFINED TETANUS TOXOID. D. H. BERGEY and S. ETRIS, J. Immunol. **31**:363, 1936.

The use of refined alum-precipitated tetanus toxoid in active immunization against tetanus is recommended. The refined toxoid had a greater antigenic value and did not produce undesirable local reactions. After two doses, a third dose

should be given at the time when an injury is sustained; in the absence of an injury the third dose should be given two years after the second. This procedure will lead to sufficient immunity against tetanus for a period of from two to three years. In the case of an injury of a nonimmunized person, Bergey and Etris recommend injection of 1,500 units of tetanus antitoxin and simultaneously a first dose of toxoid, to be followed ninety days later by a second dose. A simple test is described for the determination of at least 0.01 unit of tetanus antitoxin per cubic centimeter of blood serum. This amount of antixtoxin indicates the presence of sufficient active immunity to protect against infection with tetanus bacilli.

I. DAVIDSOHN.

THE AGGLUTINOGENS M AND N IN ALLERGIC CONDITIONS. D. HARLEY, Brit. J. Exper. Path. **17**:143, 1936.

Two hundred allergic persons were examined, and it was found that 32 per cent belonged to type M, 19.5 per cent to type N and 48.5 per cent to type MN. These percentages agree well with the results obtained by other European investigators of the distribution of the agglutinogens M and N among normal persons. Hence, Harley's findings give no indication that the properties M and N have a different distribution among allergic persons than in the general population.

A. S. WIENER.

THE ARTIFICIAL OPSONIZATION OF BACTERIA. J. GORDON and F. C. THOMSON, Brit. J. Exper. Path. **17**:159, 1936.

The work of Reiner and of Neufeld has been followed up and extended. In addition to tannic acid and the various salts studied by the previous workers, a large range of other substances has been tested for opsonic action on staphylococci. This range includes many vegetable tannins, chromium salts, iron, aluminum, lead, zinc, copper, magnesium, calcium, barium, cadmium, nickel and cobalt, and also gallic, acetic, lactic and sulfuric acids, trinitrophenol, formaldehyde and catechin. A definite parallelism has been found between tanning action and opsonic activity, and it is considered that the irreversible chemical changes characteristic of tanning are responsible for the changes in physical properties which make phagocytosis possible. Dehydration is an invariable consequence of tanning action, but the writers do not consider it to be the primary factor. The substances which show opsonic activity almost always possess agglutinating power—a property which a tannin would be expected to show on theoretical grounds.

FROM THE AUTHORS' SUMMARY.

A TRANSFUSION ACCIDENT FOLLOWING REPEATED USE OF THE SAME BLOOD DONOR. M. BAUER, Arztl. Sachverst. Ztg. **41**:1, 1935.

A woman of 34 years with lymphogranuloma, belonging to group O, was given a transfusion of 300 cc. of group O (compatible) blood. This was followed by the usual improvement in the red blood cell count and hemoglobin. Two weeks later a second transfusion from the same donor was given. The patient went into collapse immediately after the transfusion but improved after the use of stimulants. Following the transfusion, there was no hemoglobinuria, but the urine showed an increase in urobilin, and the red blood cell count fell instead of rising. There was no reaction following a third transfusion of group O blood from a different donor.

A. S. WIENER.

RELATIONSHIP OF WEATHER CONDITIONS TO POLIOMYELITIS IN THE STATE OF NEW YORK. W. F. PETERSON and FLORENCE BENELL, Bioklimat. Beiblätter **4**:160, 1935.

The physiologic status of the vascular system is influenced by changes in barometric pressure, humidity and temperature, by storms and by various other

climatic conditions. Bacteria of the upper respiratory tract possess greater power of penetration when meteorological conditions are unfavorable. Analysis of large groups of cases of poliomyelitis in Philadelphia, Chicago and especially the state of New York reveals an association between each polar episode and the outbreak of symptoms of this disease. FREDERICK STENN.

AGGLUTINOGENS M AND N. W. LAVES, Festschr. Ver. Aerzte Steinmark, 1935, p. 50.

In 2,000 tests, made mostly on syphilitic persons, the following distribution was found: type M, 30.5 per cent; type N, 18.6 per cent; type MN, 50.9 per cent. The bloods of 278 mothers and their 279 children were also tested, and not a single exception to the theory of Landsteiner and Levine was found. A. S. WIENER.

THE REACTION OF THE LUNG OF THE RABBIT IN ACUTE ANAPHYLACTIC SHOCK. R. KNEPPER and G. WAALER, Virchows Arch. f. path. Anat. **296**:465, 1935.

In the guinea-pig acute anaphylactic shock leads to spasm of the bronchial musculature and to death by asphyxia. In the rabbit also respiration ceases before the heart beat, but this is not due to bronchial constriction; the small vessels of the lung are occluded by conglutination thrombi. But this is not the only factor in the mechanism of death, for perfusion experiments in which there can be no intravascular precipitation show that the pulmonary arterial vessels are constricted. The authors believe that acute anaphylactic death in the rabbit is due to two factors, pulmonary vascular constriction and intravascular precipitation, the latter due to physiochemical changes brought about by the antigen-antibody reaction. O. T. SCHULTZ.

EFFECT OF THERAPEUTIC INHALATION OF CHLORINE ON THE FORMATION OF ANTIBODIES. J. J. LINTWAREFF, Virchows Arch. f. path. Anat. **296**:551, 1936.

Lintwareff's concept of the formation of antibodies is as follows: Toxins are adsorbed by the hemoglobin of the erythrocytes. The poisoned hemoglobin is taken up by the cells of the reticulo-endothelial system, especially by those of the spleen and liver. Here it is broken down by intracellular digestion. The freed toxin stimulates the reticulo-endothelial cells to the formation of antibodies, the liberated iron acting as a catalyst. When chlorine is inhaled in dilutions of from 1:180,000 to 1:200,000, as has been recommended in the treatment of infectious diseases, especially those of the respiratory tract, hemolysis results. Such chlorine-poisoned hemoglobin is not taken up by the cells of the reticulo-endothelial system and hence there is no stimulation to the formation of antibodies. The therapeutic inhalation of chlorine is therefore inadvisable. O. T. SCHULTZ.

THE SIGNIFICANCE OF VI ANTIGEN AND ANTIBODY IN TYPHOID FEVER. M. GUNDEL and Y. B. ABDOOSH, Zentralbl. f. Bakt. (Abt. 1) **136**:54, 1936.

Recently isolated strains of typhoid bacilli in the second or third subculture were tested for Vi antigen (Felix and Pitt, 1934). Twenty-two strains were tested, of which fourteen were isolated from the blood, four from the urine and four from the stools. Fifty-two serums were tested; twenty did not give and twenty-one gave positive Widal reactions; eleven were from persons convalescing from typhoid. Most of the freshly isolated strains contained Vi antigen, particularly strains from the blood. All the serums but one from patients with typhoid failed to give agglutination for Vi antigen, and only one of the eleven serums from persons convalescing from typhoid gave positive agglutination for Vi antigen. The authors conclude, therefore, that in most cases typhoid arises and takes its course without the development of Vi antibodies. PAUL R. CANNON.

CLINICAL SIGNIFICANCE OF TRYPTOPHAN IN THE BLOOD SERUM IN INFECTIOUS DISEASES. A. HEYMER and T. WOHLFEIL, Ztschr. f. Immunitätsforsch. u. exper. Therap. **87**:113, 1936.

Fischer's modification of Fuehrt's method was employed. In various forms of tuberculosis, in nephritis, arthritis, pneumonia and typhoid and in other acute infectious diseases, a rise of the tryptophan was observed in the acute stages of the disease. It was proportionate to the severity and intensity of the disease and parallel to the rate of sedimentation of the erythrocytes. The relation to the changes in the morphology of the blood was not as regular as that to the sedimentation rate. The authors believe that the estimation of tryptophan permits conclusions concerning the immunologic response and the prognosis.

I. DAVIDSOHN.

Tumors

MELANOMA. D. H. AFFLECK, Am. J. Cancer **27**:120, 1936.

Affleck studied 215 cases of pigmented nevi and 317 cases of malignant melanoma from the standpoint of distribution, incidence with respect to age, treatment and results. The location of the benign nevi is chiefly on exposed areas, in the following order: face, upper extremity, chest, back, lower extremity and scalp. Malignant melanoma is slightly more prevalent in males than in females. It is extremely rare in the colored race. Only 4 cases of malignant melanoma in persons under the age of 20 were recorded; the incidence between the ages of 20 and 70 was fairly uniform. In 266 instances malignant melanoma developed from a previously existing quiescent nevus and in no case from a hairy mole. That trauma is a factor in the malignant change in a quiescent nevus is suggested by the sites of predilection of malignant melanoma, namely, the foot, leg, arm, face and back. The prognosis is not dependent on the histologic type but on whether or not metastases are present at the time the malignant change is first discovered. If complete removal is effected early in the course of malignancy, there is a good possibility for a fairly long arrest. It must be remembered, however, that a clinically manifest malignant mole usually represents far-advanced malignancy and is most often hopeless. Metastasis may take place by way of the blood stream or the lymphatics. In this series metastasis by the lymphatics took place in 121 instances. The most successful method of treatment is removal of benign pigmented nevi while in the quiescent stage, particularly those in areas subject to trauma.

FROM THE AUTHOR'S SUMMARY.

HORMONAL PATHOGENESIS OF ADENOCARCINOMA OF THE BREAST. A. LACASSAGNE, Am. J. Cancer **27**:217, 1936.

If one accepts the consideration of adenocarcinoma of the breast as the consequence of a special hereditary sensibility to the stimulus to proliferative action of an estrogenic hormone, one is led to imagine a therapeutic preventive for subjects predisposed by their heredity to this cancer. It would consist—perhaps in the very near future when the nature and use of hormones will be better understood—in the suitable use of a hormone, antagonistic or excretory, to prevent the stagnation of the estrogenic hormone in the ducts of the breasts. Moreover, many other cancers, among the most frequent, seem likewise to have their origin in glandular ducts submitted to retention (for example, sebaceous, sudorific, uterine, prostatic and pharyngeal glands). If the retained products contain a sexual hormone or other organic chemically related substance, one might envisage a like pathogenic mechanism and foresee analogous attempts at prophylaxis.

FROM THE AUTHOR'S SUMMARY.

EFFECT OF ESTRIN ON THE INCIDENCE OF MAMMARY CANCER IN MICE. V. SUNTZEFF and others, Am. J. Cancer **27**:229, 1936.

The two methods by which it has been possible to prove the etiologic significance of internal secretions in the origin of cancer, namely, decreasing and increasing the action of ovarian hormones, have led to concordant results. By means of the former it has been shown that the development of so-called spontaneous mammary carcinoma in mice is due to ovarian hormones acting on mammary tissue which has been made especially responsive to such stimulation by hereditary factors; the greater the amount of hormone which is allowed to act, the greater is the effect. These experiments have also established that the greater the hereditary responsiveness of the tissue, the greater is the number of tumors which develop and the earlier they appear. The latter method has proved that through the administration of an excess of the ovarian estrogenic hormone it is possible to increase the number of cancers over that of spontaneously occurring tumors in nonbreeding mice. It has been shown also that the mammary glands of male mice are hereditarily at least as predisposed to the development of carcinoma as the female glands, as is indicated by the fact that after similar amounts of estrogenic substance have been injected into male and female mice cancers develop as readily, or perhaps more readily, in the males as in the females.

FROM THE AUTHORS' SUMMARY.

TRANSPLANTATION OF TUMORS INDUCED BY 1, 2, 5, 6-DIBENZANTHRACENE IN ALBINO MICE. M. R. LEWIS and E. G. LICHTENSTEIN, Am. J. Cancer **27**:246, 1936.

The results of the experiments show that the resistance of the Bagg albino mice to the transplantation of tumors induced in mice of the A strain by means of 1, 2, 5, 6-dibenzanthracene was broken down by frequent inoculations of pieces of the tumors to which they had at first proved refractory. Tumors of the A strain that developed in previously refractory Bagg albino mice as a result of repeated inoculations were to some extent transplantable into both the refractory strain and the strain in which the tumors originated. The repeated immediate growth and later regression of pieces of tumors from the A strain in the Bagg albino mice that remained refractory to successive inoculations of such tumors did not render these mice immune to the transplantation of tumors induced in their own strain.

FROM THE AUTHORS' CONCLUSIONS.

PRODUCTION OF MALIGNANT TUMORS IN ALBINO RATS BY ULTRAVIOLET RAYS. H. H. BEARD, T. S. BOGGESS and E. VON HAAM, Am. J. Cancer **27**:257, 1936.

Applying the technic of Roffo (twenty hours of ultraviolet irradiation daily for one year) the authors were able to produce sarcoma and carcinoma on the eyes, ears and heads of 40 per cent of their animals. Cataracts also appeared on the eyes after two months' irradiation. This technic is recommended for routine production of malignant growths in small animals without the toxic effects which may be produced by injection of carcinogenic substances. Because of the different behavior of rats and man toward sunlight and the massive doses of radiant energy necessary to produce the cancerous changes, no conclusions should be drawn regarding the carcinogenic effect of sunlight and ultraviolet rays on human beings.

FROM THE AUTHORS' SUMMARY.

SPINDLE CELL TUMORS AND LEUKEMIA IN MICE AFTER INJECTION OF A WATER-SOLUBLE COMPOUND OF 1,2,5,6-DIBENZANTHRACENE. H. BURROWS and J. W. COOK, Am. J. Cancer **27**:267, 1936.

Repeated injections of a water-soluble compound, namely, sodium 1,2,5,6-dibenzanthracene-9,10-endo-$\alpha\beta$-succinate, into mice were followed sometimes by growth of spindle cell or polymorphic cell tumors having malignant characters, sometimes by leukemia and sometimes by both of these conditions. In one instance lymphosarcomatosis occurred.

FROM THE AUTHORS' SUMMARY.

GENESIS OF GIANT CELL TUMORS. H. BERGSTRAND, Am. J. Cancer **27**:701, 1936.

An attempt is made to show that giant cell tumors are results of local processes of bone resorption. When the lime salts and the collagenous fibrils are resorbed, the original bone-forming tissue, which is a syncytium, is set free, part of it to line the lumens of the vessels and part to be differentiated as a "fibrous bone marrow." In the bone tissue thus dedifferentiated there is a tendency to proliferation, which gives rise to giant cell tumors. The giant cells are not "osteoclasts"; they are "unmasked" bone corpuscles. The tendency to proliferation gradually ceases, the giant cells disappear, and regressive changes occur in the fibrous bone marrow. A bone cyst will be the ultimate result. The giant cell tumors are thus neither inflammatory lesions nor neoplastic growths.

FROM THE AUTHOR'S SUMMARY.

LYMPHEDEMA OF THE EXTREMITIES FROM INVASION OF LYMPHATIC VESSELS BY CANCER CELLS. HAMILTON MONTGOMERY, Arch. Int. Med. **57**:1145, 1936.

In two unusual cases, lymphedema of the extremities was the result of malignant invasion of the lymphatic structures. In the first case the condition was caused by a squamous cell carcinoma extending from the left ankle to the groin. In the second case the cause probably was direct extension of endothelioma of the pleura to the lymphatic structures of one arm. Lymphedema, or elephantiasis (an advanced stage of lymphedema), of the extremities rather frequently results from metastatic involvement of inguinal or axillary lymph nodes or from surgical removal of lymph nodes, especially those which are involved in carcinoma of the breast. Development of lymphedema as the result of packing of the lymphatic channels of an extremity with tumor cells, thus obstructing the flow, is believed uncommon.

FROM THE AUTHOR'S SUMMARY.

GLIOMATOSIS OF THE LEPTOMENINGES. S. MAHON, Arch. Neurol. & Psychiat. **35**:1309, 1936.

Of ninety-two cases reported in the literature as instances of diffuse sarcomatosis of the leptomeninges, only nine may be accepted, according to Mahon, as instances of sarcomatosis. In twelve cases, a primary tumor was absent, and the diffuse infiltration appeared as milky thickening of the leptomeninges. In two of these twelve cases the diagnosis of gliomatosis was definite. Mahon reports a thirteenth case in which a primary tumor was lacking and which he thinks is a third case of leptomeningeal gliomatosis. The patient, aged 30, had been suffering from paroxysmal attacks of headaches, convulsions and disturbances of vision, smell and speech. There was secondary atrophy of the optic nerve. Death followed ventriculography. Necropsy revealed a tense dura, cerebral herniations, and milky thickening of the leptomeninges over the inferior surface of the frontal lobe and the lateral convexities of the hemispheres and cerebellum. A tumor was observed in the cortex of the right lateral border of the cerebellum and another (a nodule, 1 cm. in diameter) in the roof of the right lateral ventricle, beneath the ependyma. The third ventricle was partly occluded by tissue growth. Metastatic tumor cells were numerous in the subarachnoid space in sections from the cerebellum, sylvian aqueduct and inferior cerebellar surface. The cells both in the macroscopic nodules and the meninges were probably medulloblasts or spongioblasts, though the cerebellar tumor was not medulloblastoma, glioblastoma multiforme or spongioblastoma polare. Mahon could not explain the relationship between the macroscopic nodule of the cerebellum, the leptomeningeal thickening, the ependymal changes and the foci of glial proliferation over the surface of the cerebellum. It is suggested that the term "sarcomatosis of the meninges" should be discarded when one deals with invasion of the leptomeninges by small round cells.

G. B. HASSIN.

EFFECTS OF EXHAUST GASES FROM INTERNAL COMBUSTION ENGINES AND OF TOBACCO SMOKE ON MICE, WITH SPECIAL REFERENCE TO INCIDENCE OF TUMORS OF THE LUNG. J. A. CAMPBELL, Brit. J. Exper. Path. **17**:146, 1936.

Mice were exposed each week for seven hours on each of five days during most of their lives to exhaust gases from internal combustion engines in concentrations somewhat resembling those that obtain in traffic blocks and garages. There were no marked effects on the well-being of the mice. Carbon (soot) and lead were excluded in these experiments. Mice were similarly exposed to fumes and tarry matter from cigaret smoke, without marked pathologic effects. The incidence of primary tumor of the lung in mice is considered in detail. It is usually rare for such a tumor to occur before the latter half of the second year of life. Exposure to exhaust gases has little effect on the incidence of tumor of the lung in mice. The incidence for the tobacco group of mice is on the high side, but these mice survived longer than the other groups. The incidence is increased by dust collected from tarred roads, as shown previously. Some of these tumors are definitely malignant in appearance. It is not known yet whether the dust freed from tar has any influence; this question is under examination, and the results should be available in a few months. Age and heredity have an influence on the incidence of these primary tumors in the lungs of mice but are not responsible for the result with the tar dust. This dust contained much more tar than the tobacco smoke.

FROM THE AUTHOR'S SUMMARY.

ELIMINATION OF 1, 2-BENZPYRENE, 1, 2, 5, 6-DIBENZANTHRACENE AND ANTHRACENE FROM THE BLOOD STREAM. P. R. PEACOCK, Brit. J. Exper. Path. **17**:164, 1936.

When intravenously injected into rabbits, guinea-pigs and fowls, colloidal 1, 2-benzpyrene, 1, 2, 5, 6-dibenzanthracene and anthracene disappear rapidly from the circulating blood. The white cells of the blood are not responsible for this disappearance. Study of frozen sections of various organs shows that the liver contains intracellular globules of lavender fluorescence following intravenous injection of benzpyrene colloid. Samples of bile obtained by cholecystostomy show blue-violet fluorescence shortly after intravenous injection of benzpyrene or anthracene colloid but not after that of dibenzanthracene colloid. The fluorescence with benzpyrene colloid is not due to this substance but to a fluorescent derivative or derivatives showing ill defined fluorescent bands with maxima at, approximately, 4200 and 4550 angstroms. This substance has not been recovered from excreta, but unchanged benzpyrene in solution has been found occasionally in excreta after intravenous injection of large amounts of the colloid. In rabbits the urine after intravenous injection of benzpyrene, dibenzanthracene and anthracene colloids contains an ether-soluble substance or substances with blue fluorescence, not normally present. Fluorescence spectra of extracts of such urine show no characteristic bands.

FROM THE AUTHOR'S SUMMARY.

NEUROGENOUS TUMORS OF THE DIGESTIVE TRACT. F. FEYRTER, Virchows Arch. f. path. Anat. **295**:480, 1935.

As a rare finding Feyrter describes four examples of circumscribed small nodular growths of the stomach, small intestine and colon. They are composed of short plump spindle cells with granular cytoplasm and of granular syncytial strands, the latter directly continuous with Meissner's plexus. The cells and syncytial tissue are held to be derivatives of the sheath of Schwann. The tissue also contains large vacuolated cells, similar to Gaucher cells; their origin and nature remain undetermined. Feyrter proposes the name "granular cell neurogenous growths of the digestive tract." (The growths described are apparently identical with the diffuse or localized hyperplasias of the intrinsic nerve tissue described by Masson and seen by many in the appendix.)

O. T. SCHULTZ.

ENDOTHELIOMA OF THE PLEURA. T. FAHR, Virchows Arch. f. path. Anat. **295**: 502, 1935.

After a brief introductory discussion of the differences of opinion that still exist relative to the origin and nature of the so-called pleural endothelioma, Fahr describes seven examples of this form of neoplasm. As the result of personal examination of primary carcinoma of the lung in 203 persons in the past decade, he cannot accept Robertson's view that the pleural growth is primary carcinoma of the lung invading the pleura diffusely. The condition under consideration is characterized by marked diffuse fibrous thickening of the pleura, which leads to compression of the lung and is associated with a hemorrhagic pleural transudate. The striking histologic feature is the marked overproduction of collagenous connective tissue, in which are embedded carcinoma-like cellular alveoli. As a result of his study Fahr maintains that growths of this description are derived from the surface cells of the pleura. The collagenous tissue is formed directly by the cellular derivatives of the surface cells, and the growth is therefore a mesenchymal neoplasm in which the parenchymal cells form a collagenous ground substance. He proposes the designation "diffuse fibroendotheliomatosis of the pleura," with the understanding that "endothelium" refers to the surface cells of the pleura. The pleural neoplasm is to be clearly distinguished from primary carcinoma of the lung. A benign form of the pleural growth has not been described.

O. T. SCHULTZ.

Society Transactions

PATHOLOGICAL SOCIETY OF PHILADELPHIA

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Dec. 10, 1936

HERBERT L. RATCLIFFE, *Secretary*

SILICOSIS AND RELATED CONDITIONS (ANNUAL GROSS LECTURE). LEROY U. GARDNER.

The economic importance of silicosis was not recognized until South Africa was attempting to rehabilitate itself after the Boer War. In the United States the condition had been studied by Landis and Pancoast in sanatorium patients from anthracite mines and potteries, but the lead and zinc miners of the Joplin, Mo., district were the first large mining group which was intensively studied. The depression was responsible for the popularization of knowledge of silicosis in this country.

Modern concepts of the disease are based on South African experience. They were formulated for various industries by Collis, who showed that silica was the dangerous substance common to mining, grinding and the potteries. He believed that the silica in the dust must be in free crystalline form and not combined in a silicate, and that it must be fine enough to stay in suspension and to be inhaled. He pointed out that its action was not mechanical but probably physicochemical, and that it predisposed to tuberculosis, which often remained latent, usually not giving rise to infection by contact. More recent work has indicated, however, that one form of combined silica, the mineralogical group known as asbestos, also is capable of causing pulmonary fibrosis, and unreported experiments show that free silica in an amorphous state (opal and diatomaceous earth) causes progressive fibrosis when injected into animals.

Silica, in contrast to other minerals, stimulates pronounced activity of mononuclear cells in the lungs. These cells take up the silica and transport it to the lymphatic nodes, where they accumulate in groups, stimulating growth of nodules of hyaline fibrous tissue. When these nodules are sufficiently large and numerous, lymph drainage is impaired, and silica is transported to the fibrous tissue about the lymphatic vessel, where linear fibrosis occurs, and other nodules are formed. Later, phagocytic cells accumulate in the parenchyma, additional nodules develop, and alveolar walls are thickened.

Common complications of silicosis are emphysema, pleurisy, possibly heart disease and infection. As a cause of the last tubercle bacilli are most frequent. The anatomic manifestations of tuberculosis are discussed with special reference to silicotuberculosis, a chronic state resulting from the combined action of silica and the tubercle bacillus. The mechanisms by which silicosis may affect tuberculous infection are reviewed.

The reactions to other types of dust are of low intensity and characteristically linear because of the distribution about lymphatic vessels.

CHICAGO PATHOLOGICAL SOCIETY

CARL W. APFELBACH, *President*

Regular Monthly Meeting, March 8, 1937

EDWIN F. HIRSCH, *Secretary*

THE SO-CALLED SUPERIOR PULMONARY SULCUS TUMOR. I. DAVIDSOHN and L. FELDMAN.

The term "superior pulmonary sulcus tumor" was introduced by Pancoast in 1932 to designate a tumor producing a complex characterized by: the presence of the tumor at the thoracic inlet; pain about the shoulder and down the arm; Horner's syndrome (unilateral ptosis, miosis, enophthalmos and anhidrosis); atrophy of the muscles of the arm, and, in roentgen films, a small homogeneous shadow at the extreme apex of the lung, with evidence of local destruction of ribs and often infiltration into the adjacent vertebrae. Pancoast based his conclusions on a study of seven patients. Four of them he had reported on in 1924. None was examined post mortem, and only two had biopsies. One of these biopsies yielded a diagnosis of spinocellular carcinoma; the other, a diagnosis of metastatic carcinoma. Pancoast assumed that a tumor of the superior pulmonary sulcus develops from a congenital bronchiogenic cyst of the fifth pharyngeal pouch. A review reveals no justification for this assumption. Any tumor that involves the brachial plexus and damages the cervical sympathetic nerve will cause the symptoms and changes which Pancoast considered characteristic for tumor of the superior pulmonary sulcus.

Reports of sixteen cases in which necropsy was done were found in the literature, namely, thirteen cases of primary bronchiogenic carcinoma, one case of sympathoblastoma of the mediastinum, one case of carcinoma of the thymus and one case, reported in 1838, of "glandular scirrhus" of the neck. We report a case of primary bronchiogenic colloid adenocarcinoma of the apex of the right lung in a man aged 31 years, who lived about one year after the onset of symptoms. He had the clinical and roentgenologic findings of the so-called tumor of the superior pulmonary sulcus. The tumor infiltrated the brachial plexus and the wall of the chest and destroyed parts of the first, second and third ribs and the first, second, third and fourth thoracic vertebrae. Metastases were found in the mediastinal and retroperitoneal lymph nodes, pancreas, adrenal glands and kidneys. Bronchopenumonia and an abscess involved the upper lobe of the left lung. Old calcified tubercles were present in the mediastinal lymph nodes, spleen and liver.

A complex of pain in the shoulder and arm, Horner's syndrome and atrophy of the muscles of the arm with roentgen evidence of a mass at the extreme apex of the lung and of local destruction of ribs and the adjacent vertebrae is not due to a specific type of tumor, as was postulated by Pancoast; it may be due to various types with different origins. A review of the published reports indicates that a primary bronchiogenic carcinoma has been the tumor most frequently associated with this complex.

DISCUSSION

R. E. MALCOMB: Was the brain examined, and do you think that the metastases to the kidneys had spread along fascial planes?

P. STEINER: Is the fibrous region in the apex of the left lung a tuberculous scar?

H. SWEANY: Pulmonary carcinoma may not give the impression of a growth of tumor cells into the finer structures of the lung. Several years ago I reported a carcinoma with definite growth along the alveoli.

O. SAPHIR: Colloid carcinoma of the lung may arise from the mucous glands in the wall of a bronchus. This tumor may have had such an origin.

I. DAVIDSOHN: The brain showed only edema. There is no satisfactory explanation as to why the usual lymph channels were not followed in the metastatic spread of this carcinoma. Coexisting tuberculosis has been noted in many cases of this type. Probably the usual lymph channels were blocked by the inflammation. I did not wish to imply that the carcinoma had spread along the alveolar walls of the lung. There was simply a resemblance to alveolar structure in certain portions. Colloid carcinoma of the lung may arise from the lining cells of a bronchus as well as from the mucous glands in the wall.

HYDRONEPHROSIS IN A SUPERNUMERARY KIDNEY. HAROLD LAUFMAN.

Anomalies of the kidney result from a complication of the embryologic development. A review reveals only thirty-seven reports of cases accepted as examples of true supernumerary kidney. This condition should not be confused with fused or double kidney. Only three other cases of hydronephrosis of a supernumerary kidney have been reported.

A woman aged 56 years had headache and fever for three days. A nontender mass in the right upper quadrant of the abdomen displaced the right kidney upward. The left kidney was normal. The sediment from centrifugated urine contained 20 leukocytes per field (high magnification). A mass 7.5 by 4 cm., with a small kidney attached above, was removed. This mass was a pyelectatic cyst with several thin-walled accessory cysts and a ureter dilated to 2.5 cm. The latter was joined at the ureteropelvic junction by the normal ureter from the upper kidney. Microscopic sections of the wall of the large cyst contained renal parenchyma with a few glomeruli, distorted tubules and interstitial fibroplastic stroma. The upper kidney, though small, had a normal histologic structure. The two kidneys were anatomically entirely separate, being joined only by a fibrous and areolar tissue band.

DISCUSSION

O. SAPHIR: Simple duplication of the ureter and renal pelvis must not be confused with supernumerary kidney. With true supernumerary kidney there are only fibrous tissues, not renal tissues, between the two structures.

ALLERGIC LESIONS PRODUCED BY INJECTION OF PROTEIN. LOUISA HEMKEN and G. J. RUKSTINAT.

In an effort to become familiar with the changes induced in endothelium by the allergic response to horse serum, detailed studies were made of the histologic changes in the arteries of the heart, lungs, spleen, kidneys and adrenal glands in rabbits. The endothelial proliferation and granulation tissue formations in these vessels were directly proportional to the amounts of foreign protein injected. The most marked changes were in the coronary and pulmonary arteries and were characterized by intimal thickening, occasionally with palisade orientation of the cells. The arteries of the second and third order in the tissues were most markedly affected.

The changes were not specific but were characterized by a combination of intimal proliferation, medial necrosis and localized adventitial inflammation. The alterations were similar to those in periarteritis nodosa and in rheumatic endocarditis and carditis. Swelling of the endocardium, especially of the coverings of the mitral valve, bore a marked resemblance to the changes induced by pitressin (beta hypophamine). The common factor in the production of similar lesions by widely different agents emphasizes the participation of the reticulo-endothelial system in such reactions.

DISCUSSION

I. PILOT: Were the changes due to toxin rather than to allergy?

O. SCHULTZ: A fibrinoid reaction is considered characteristic of allergy.

H. SWEANY: The lesions of periarteritis nodosa are considered by some to have an allergic basis.

G. RUKSTINAT: The vascular lesions were beaded somewhat like the lesions in periarteritis nodosa.

THE VARIABILITY OF THE TUBERCULIN REACTION. JOHN HOWE.

Daily intracutaneous tuberculin tests with simultaneous use of two different dilutions of purified protein derivative were made on four patients with clinical tuberculosis and two control subjects with positive reactions to tuberculin but without clinical evidence of tuberculosis. The shortest period of observation was one month; the longest was over four months. The average diameter of the reactions was read twenty-four hours after injection. The von Gröer products for these readings were calculated as the result of multiplying the average diameter in millimeters by the negative logarithm of the tuberculin concentraton. These products were then plotted on a graph in which the base line was the date of injection, to make a curve representing the course of allergy in each subject.

The curves of allergy for all subjects studied show marked fluctuation in the reaction to the same dose of the purified protein derivative in the same subject on different days. This fluctuation recurs more or less regularly at intervals of from one to several days. The range of fluctuation, calculated in percentages of the maximal tuberculin reaction, is from 50 to 100 per cent in the patients and from 55 to 67 per cent in the control subjects.

When the curves of the tuberculin reaction are compared with the curves of daily blood pressure in the same subjects, taken under basal conditions, a definite correlation is apparent. The increase in the reactions to tuberculin corresponds closely with the fall in diastolic blood pressure following a definite pressor episode; that is, with vasodilatation following vasoconstriction. This correlation varied from 77 to 100 per cent in this series of subjects. The converse is also true, namely, that periods of decreased or negative reaction to tuberculin correspond to periods of increasing diastolic pressure or vasoconstriction.

LOS ANGELES PATHOLOGICAL SOCIETY

EMIL BOGEN, *President*

March 9, 1937

O. B. PRATT, *Secretary*

TUBERCULOSIS OF THE APPENDIX. MORRIS SPECTOR.

Routine sections of 140 appendixes removed at operation from patients at the Olive View Sanatorium during the past decade revealed tuberculous lesions in 47, or 33 per cent. Acute purulent inflammation was also observed in 20 of the tuberculous appendixes, as well as in an equal number of nontuberculous ones. Most of the appendixes showing simple acute appendicitis were from men with relatively limited pulmonary lesions, while more than two thirds of the tuberculous appendixes were from women with far advanced pulmonary tuberculosis and sputum containing the bacilli. Blood counts made prior to operation in patients with normal appendixes usually showed less than 10,000 white blood cells, while in the majority of those with acute appendicitis, with or without associated tuberculosis of the appendix, the white cell counts were in excess of 15,000. The majority of the patients with simple tuberculosis of the appendix or with tuberculous peritonitis had total white cell counts between these figures. Similarly, most of the patients with normal appendixes showed less than 70 per cent polymorphonuclear neutrophils, most of those with acute appendicitis had more than 80 per cent, while those with tuberculosis of the appendix averaged 75 per cent. A shift to the left was found most marked in the patients with acute appendicitis, and less marked in those with uncomplicated tuberculosis of the appendix, while it was usually absent in those with normal appendixes. The monocyte and eosinophil counts showed no consistent relationship to the type of appendical disease.

The mortality in institutional patients with normal appendixes was less than 1 per cent, that in patients with acute appendicitis about 10 per cent and that in patients with simple tuberculosis of the appendix about the same, but more than 30 per cent of those with acute appendicitis complicating tuberculous appendicitis died.

TUBERCULOSIS OF THE TONSIL. E. LAWRENCE HAWKINS.

Routine sections of 225 pairs of tonsils removed from patients at the Olive View Sanatorium during the past decade revealed tuberculous lesions in 34, or 14 per cent. Although the surgeons, for obvious reasons, operated more often on patients with childhood, minimal or moderately advanced forms of the disease, more than 85 per cent of the tuberculous tonsils came from patients with far advanced tuberculosis. Tuberculosis of the tonsil was rarely recognized clinically in these patients, as the operation is not performed in any patient showing gross ulceration of the pharynx extending over the tonsil, or other visible tuberculous lesion, although destructive diathermy has been used in some instances of this type. One instance of miliary tuberculosis of the tonsil was found before the clinical diagnosis of miliary tuberculosis could be made, but in another instance a tonsil with only a small tuberculous local lesion was removed immediately prior to the development of a miliary spread. Other ill effects were rarely noted in this series.

MONILIAL MENINGITIS. EMIL BOGEN and JOHN KESSEL.

Infections of the brain and meninges with fungi of a number of different types have been well recognized, particularly infections with blastomycetes, coccidioides and torulas. Intracranial infection with monilas, however, is extremely rare. In the latter part of April 1936 a young unmarried woman of 25 suffered an acute febrile attack of headache together with paralysis of the sixth cranial nerve on the left side, resulting in diplopia, and disturbance of sensation and smell on the right side of the face and nose. Other findings were essentially negative. For four years she had been under institutional treatment for minimal pulmonary tuberculosis; hysterectomy and bilateral salpingectomy had been done in the preceding year, following pelvic infection; occasional otitis externa and otitis media had occurred during the previous two years. Spinal punctures on April 26, May 2 and May 6 showed clear fluid with 270, 320 and 280 cells, respectively, mainly lymphocytes but no organisms. Specimens of spinal fluid taken on May 11, May 19, May 25 and June 8, showed 119, 200, 80 and 42 cells, respectively, but these four specimens all showed a yeastlike organism, which was at first suspected of being present through contamination. Since it was present repeatedly in smears and in cultures on Sabouraud's medium, and since no such organism could be found in the control tubes, stains, water or other laboratory materials, it was finally recognized as being the infective agent. Culture and inoculation into animals showed that it was a yeast giving the cultural and fermentative reactions of Monilia psilosis, an organism pathogenic for rabbits but not for monkeys. Accordingly, beginning May 24, she was given massive intravenous injections of 5 per cent solutions of sodium iodide, up to 120 cc. per day, with marked effect. By June 20 a slight weakness of the left rectus lateralis was the only remaining evidence of the disease, and this disappeared within the next few weeks. Oral administration of a preparation of soluble iodine was continued for the next six months, and the patient was discharged as apparently completely recovered in January 1937.

Book Reviews

Chondromalacia Patellae. By Axel Øwre, Pathologic Institute, Rikshospital, Oslo (Prof. Francis Harbitz). Paper. Pp. 157, with 21 illustrations. Oslo: Nationaltrykkeriet, 1936.

The term "chondromalacia patellae" as used by the author indicates degenerative changes in the articular cartilage of the patella which may precede and always accompany degenerative arthritis of the knee joint. A complete study of the pathologic changes and later a correlation with the clinical symptoms are presented. The pathologic study includes the results of examination of the patellae of patients in five age groups, beginning with those younger than 20 years and ending with a group from 60 to 80 years of age. The author has described edema of the cartilage as probably the earliest change which can be detected microscopically. Later there may be longitudinal streaking of the cartilage, and swelling and thickening of the synovia about the cartilage. The incidence and degree of the pathologic changes and clinical symptoms increase with age. The author concludes that chondromalacia patellae most frequently shows itself during the age period from 20 to 35 years, when the degenerative changes in the patellar cartilage become more common. The clinical signs and symptoms characterizing this condition are: palpable fissure formation in the cartilage; crepitation when the patella is moved from side to side, and pain on pressure over the patella when it is pushed toward the medial femoral condyle. Capsular tenderness confined to the infrapatellar layer of fat and to the part of the capsule medial to the patella is also a suggestive symptom. Effusion into the knee is rare in the younger age groups and when present is of transient nature. The demonstration of fragments of cartilage in fluid obtained by aspiration of the joint is proof of cartilaginous destruction, but it does not permit the conclusion that chondromalacia is the sole pathologic condition obtaining. The symptom of catching or partial locking and quickly releasing is characteristic. Soreness in the knee joint after sitting for some time in a cramped position and on descending hills is a common symptom. The treatment of chondromalacia should include rest, complete immobilization, supportive bandaging and diathermy for the relief of pain. Operative treatment is recommended only if the condition is so recurrent that the patient has become more or less an invalid.

De l'effet des rayons X sur les os longs en development et sur la formation de cal. Étude radiobiologique et anatomique chez le rat. By Bjarne Dahl. Paper. Pp. 149, with 45 illustrations. Oslo: Jacob Dybwad, 1936.

This monograph of 149 pages with 38 figures and 7 pages of roentgenographic and photomicrographic prints, bound in paper, includes an excellent review of the literature in which are found reports of necrosis or other changes in bones attributed to roentgen irradiation.

The authors report in detail an extensive number of experiments in which they used rats to demonstrate the effects of the application of various amounts of roentgen radiation. Studies were made both with very young and with young adult rats. A marked individual variation was found in the response of these animals to irradiation, but there was a surprising degree of similarity between the skin reaction in a given animal and the changes noted in the bones. These changes varied from simple hyperemia in the osseous tissue irradiated to cicatrization, devitalization and necrosis.

Conclusions were based on roentgenographic, macroscopic and microscopic studies and included an attempt to determine the effect of irradiation on bone, cartilage and the individual cell elements.

This monograph represents an attempt to evaluate the known data on the entire question of changes in the skeleton produced by irradiation and contributes addi-

tional information from the author's own experimental studies. All who are interested in the growth and development of bone, as well as those who are concerned primarily with the clinical and therapeutic use of the roentgen ray, will wish to read this report.

Aids to Pathology. By Harry Campbell, M.D., F.R.C.P., and Kenneth Campbell, O.B.E., M.B., F.R.C.S. Edition 7. Price, $1.50. Pp. 263. London: Bailliere, Tindall & Cox, 1936.

This little volume has been designed primarily for students as a review of pathology in preparation for examinations. Its popularity is attested by the fact that it has run through seven editions since 1907. Despite its small compass, it covers the subject well, the material being presented in a terse and lucid style and illustrated by a few simple black and white sketches, most of which, however, are not illuminating. A small number of minor but inexcusable errors have crept into the text. For example, it is stated on page 100 that "scirrhus is the commonest form, constituting about 95 per cent of all cases of carcinoma of the breast." In a book of this character, truth is often sacrificed for the sake of simplification. Some subjects that are still sub judice are treated dogmatically as established beyond debate. Thus, the statement on page 20, "The reticulo-endothelium is the mother tissue of all the blood cells," would hardly be acceptable to the dualistic school in hematology. The book lacks an account of a number of recent commendable advances, particularly in the fields of vitamin deficiencies, virus diseases and endocrine disorders, as well as the modern views on Bright's disease, aleukemic reticulosis, Warburg's work on the aerobic glycolysis of malignant tissue, Hand-Schüller-Christian disease, Masson's glomus tumor and von Gierke's disease, to mention only a few in passing. Surely in spite of the exigencies of space, these might have been included, especially since the authors desire to bring the text up to date. Nevertheless, the book qualifies fully as a readable survey, serving the prime purpose of preparation for examinations in pathology. And examiners must be faced.

Books Received

THE AVITAMINOSES. THE CHEMICAL, CLINICAL AND PATHOLOGICAL ASPECTS OF THE VITAMIN DEFICIENCY DISEASE. Walter H. Eddy, Ph.D., Professor of Physiological Chemistry, Teachers College, Columbia University, and Director of Bureau of Foods and Sanitation, Good Housekeeping Magazine, and Gilbert Dalldorf, M.D., Pathologist to the Grasslands and Northern Westchester Hospitals Westchester County, New York. Cloth. Price, $4.50. Pp. 338, with 29 illustrations. Baltimore: Williams & Wilkins Company, 1937.

DICTIONNAIRE DES BACTÉRIES PATHOGÈNES POUR L'HOMME, LES ANIMAUX ET LES PLANTES. Dr. Paul Hauduroy and Dr. Ch. Ehringer, pour la bactériologie humaine; Prof. Ach. Urbain and Dr. G. Guillot, pour la bactériologie animale, and Dr. J. Magrou, pour la bactériologie végétale. Cloth. Price 140 francs. Pp. 598. Paris: Masson & Cie, 1937.

EL GROUPO INDETERMINADO DE LAS AFFECCIONES MALIGNAS DE LOS GANGLIOS LINFÁTICOS (LINFOGRANULOMATOSIS ATÍPICA). N. Puente Duany. Pp. 94. Havana, Cuba: A. Sanchez Velosa, 1937.

REPORT OF THE PERMANENT COMMISSION ON BIOLOGICAL STANDARDISATION. SESSION HELD AT GENEVA ON SEPTEMBER 30 AND OCTOBER 4, 1935. LEAGUE OF NATIONS, QUARTERLY BULLETIN OF THE HEALTH ORGANISATION, SPECIAL NUMBER, NOVEMBER 1936. Paper. Price, 65 cents. New York: Columbia University Press, 1937.

INDEX TO VOLUME 23

The asterisk (*) preceding the page number indicates an original article in the Archives. Subject entries are made for all articles. Author entries are made for original articles and society transactions. Book Reviews, Obituaries and Society Transactions are indexed under these headings in alphabetical order under the letters B, O and S, respectively.

Abnormalities and Deformities: See also under names of organs and regions, as Arteries, abnormalities; Brain, abnormalities; Heart, abnormalities; Liver, abnormalities; etc.
duplication of congenital malformations in brothers and sisters and among other relatives, 886
Abscess: See under names of organs and regions, as Brain, abscess; Lungs, abscess; etc.
Acid, Amino: See Amino-acids
chemical reaction between oleic acid and aqueous solutions of magnesium, 752
Actinomycosis, actinomycotic abscess of brain, *58
Bacterium necrophorum from chronic ulcerative colitis, further studies, 453
of tubes and ovaries, *53
Adams, W. E.: Experimental production of bronchiectasis in dogs, 606
Addison's Disease, histology of homoplastic adrenal transplant that survived for 8 months in patient with Addison's disease, 141
pathologic changes in adrenalectomized dogs, particularly in anterior lobe of hypophysis, 579
Adenocarcinoma, enzyme content of parenchymatous adenocarcinoma of pancreas, 135
of breast, hormonal pathogenesis of, 900
Adenoma, change in basophil cells of pituitary gland in syndrome attributed to basophil adenoma, 279
subclinical, of pituitary gland, 289
Adipose Tissue: See Fat; Lipoma; Obesity
Adolescence, influence of thymus extract on certain endocrine organs of rat, 293
Adrenalectomy: See Adrenals
Adrenals, alterations in lipoid content in infancy and in childhood, 718
and thymus in response of organism to injuries and intoxications, 580
changes induced by estrogens in male mice, 581
cortex, 882
cystic lymphangioma of, 137
effects of adrenalectomy and hypophysectomy on experimental diabetes in cat, 276
histology of homoplastic adrenal transplant that survived for 8 months in patient with Addison's disease, 141
necrosis with calcification of, 584
pathologic changes in adrenalectomized dogs, particularly in anterior lobe of hypophysis, 579
rôle of adrenal cortex in production and utilization of vitamin C; its influence on structure of teeth in albino rat, *372
Agglutination, blood group ferment in saliva, 592
capacity of boiled erythrocytes to remove agglutinins, 132
group-specific A substance in commercial pepsin, 896
identity of precipitin and agglutinin, 895
Agglutinins: See also under Antigens and Antibodies; Blood, groups; Immunity; Tuberculosis; etc.
for sheep erythrocytes in human blood, 443
Agglutinogens: See Agglutination; Blood, groups
Agranulocytosis: See Angina, agranulocytic
Ahronheim, J. H.: Size of spleen and liver-spleen ratio; statistical study based on 1,000 autopsies, *33
Alcohol, factors influencing fatty condition in dog's liver produced by alcohol and diet rich in fat, 740
Alford, J. E.: Congenital bronchogenic cyst of mediastinum in infant, 296
Allergy: See Anaphylaxis and Allergy
Alum: See under Poliomyelitis
Amebae, contamination of bacterial culture by organism resembling Hartmannella Castellanii, 129
Ameloblastoma, histologic study of, *664
survey of 379 cases from literature, *831
American Association of Pathologists and Bacteriologists, annual meeting, 275, 576
Association of Public Health, meeting, 713
Leprosy Foundation, 713
Society of Clinical Pathologists, meeting, 430
Amido-Azotoluene, experimental carcinoma of liver, 596
Amino-acids and staphylococcus toxin, 133
Amyloidosis, corpora amylacea in nerve cells, 720
Anaphylaxis and Allergy: See also Tuberculosis, allergy in; etc.
agglutinogens M and N in allergic conditions, 898
allergic lesions produced by injection of protein, 907
anaphylaxis in frogs, 134
desensitization of passively sensitized guinea-pigs, 133
development of sensitization to foreign protein in human beings, 730
effect of nerve stimulation on hyperergic inflammatory reaction, 123
hyperergic reaction of denervated kidney, 581
inadequacy of allergic inflammation as protection against infection of rabbits with virulent pneumococci, 145
local reactions from intradermal injection of small amounts of antiserum, 728
localization of hyperergic inflammatory reaction, 883
phenomenon of local skin reactivity to bacterial filtrates, 891
reaction of lung of rabbit in acute anaphylactic shock, 899
rôle of allergy in pathogenesis of progressive thrombosis especially in regard to changes in endothelial lining of large peripheral veins, *501
studies in synergy, 895
tuberculous liquefaction, 442
Anemia, bone marrow as studied by sternal biopsy, 292
influence of infection and intoxication on hemoglobin production in, 580
Leuko-Erythroblastic: See Hematopoietic System
macrocytic, correlation of serum iron, bone marrow and blood cell changes following specific therapy in, 738
pernicious, sternal marrow in; correlation of observations at biopsy with blood picture and effects of specific treatment in megaloblastic ("liver-deficient") hyperplasia, *159
primary lymphosarcoma of liver with metastases to marrow and secondary anemia, *674
sickle-cell, new cause of cor pulmonale, 277
Aneurysm, aortic; aneurysm of sinus of Valsalva with rupture into right auricle, *679
cerebral; coarctation of aorta with intermittent leakage of congenital cerebral aneurysm, 884
Angina, agranulocytic; changes in bone marrow in agranulocytosis, 277
Ludwig's, due to esculin-fermenting hemolytic streptococcus (enterococcus), 295
Vincent's; significance of fusiform bacilli and spirilla in gingival tissue, 723

Angina Pectoris, genesis of multiple encephalomalacia and of angina pectoris in foam formation, 280
Angioma, Kaposi's disease as systemic angiomatosis, 736
Anoxia: See under Oxidation reduction
Anthracene, elimination of 1,2-benzpyrene, 1,2,5,6-dibenzanthracene and anthracene from blood stream, 903
Anthracosis: See Pneumoconiosis
Antibodies: See Antigens and Antibodies
Antigens and Antibodies: See also Agglutinins; Immunity; Lipoids; Precipitin
antibodies for Trichomonas foetus, 744
antigenic composition of virus of psittacosis, 731
antigenic function of kidney, 591
antigenicity of carbohydrates, 287
detection of antigenic differences in mouse erythrocytes by means of immune serums, 441
heterophile hapten and specific polysaccharide hapten of smooth form of Bacterium Shigae, 285
inactivation of H antigen by dilute mineral acid, 132
localization and concentration of antibodies and colloidal dye in areas of inflammation of various ages, 145
significance of Vi antigen and antibody in typhoid fever, 899
synergy of antibodies, 442
tumor antigens, 597
Vi antigens of various Salmonella types, 441
Antiserum: See under names of various diseases, as Diphtheria; etc.
Antistreptolysin: See Streptococci
Antitoxin: See under Diphtheria; Tetanus; etc.
Aorta, Aneurysm: See Aneurysm, aortic
coarctation, with intermittent leakage of congenital cerebral aneurysm, 884
necrosis of arteriosclerotic plaques and its relation to atheroma, 586
origin of 1 coronary artery from pulmonary artery, 435
relation between diameter of aorta and distribution and extent of atherosclerosis, 888
variations in structure of normal human aorta, 436
Aortic valve, so-called congenital bicuspid, *350
Apfelbach, C. W.: Modern concepts of cirrhosis of liver, 142
Apparatus, hydraulic automatic tissue changer or immersion device, *392
procedure and apparatus for preservation in lyophil form of serum and other biologic substances, 448
simple mailing container for slides, *849
simple small paraffin ovens or constant temperature baths constructed at small cost, *539
Appendix, melanosis of colon, 610
tuberculosis of, 908
Arhinencephalia: See Brain, abnormalities
Arrhenoblastoma, ovarian tumors (granulosa cell carcinoma, arrhenoblastoma, dysgerminoma, Brenner tumor), 288
Arsenic, karyoclastic poisons, 887
Arsphenamines, death of 2 syphilitic infants from hemorrhagic encephalitis following treatment with sulpharsphenamine, 431
hypersensitiveness of guinea-pigs to, 591
Arteries: See also Aneurysm; Aorta; Arteriosclerosis; Blood pressure; Blood vessels; Embolism; Periarteritis; Thrombosis; etc.
abnormalities; so-called congenital absence of left coronary artery in child, 294
ash content of normal and pathologic large arteries, 727
changes in appearance of wall of muscular artery between diastolic and systolic blood pressures, 884
coronary; experimental coronary occlusion, 577
fat deposition in arteries treated in vitro with B factor, *332
origin of 1 coronary artery from pulmonary artery, 435
Arteries—Continued
pulmonary; so-called primary sclerosis of pulmonary vessels, 126
ridges and cell buds of arteries of thyroid, 127
transverse intimal striation of arterial system, 585
Arteriosclerosis, Aortic: See under Aorta
mechanical factors in, *1
renal; benign and malignant hypertension and nephrosclerosis, 431
Arthritis, acid-fast and alcohol-fast bacilli in urine of patients with rheumatic polyarthritis, 894
chronic, in lower mammals, 293
classification based on pathologic observations in joints, 752
Asbestosis: See Pneumoconiosis
Atheroma, necrosis of arteriosclerotic plaques and its relation to atheroma, 586
Atherosclerosis: See Arteriosclerosis
Atrophy: See Brain, atrophy; Liver, atrophy; etc.
Auerbach plexus, intestinal obstruction in infant due to unusual cause, 721
Automobile Exhaust Gas: See Carbon monoxide
Autopsies, postmortem discoloration of iris, 598
technic for removal of organs of neck, 599
Avitaminosis: See under Vitamins
Azo-Blue: See under Streptococci

B C G: See Tuberculosis
Bachem, A.: Time factor in irradiation of malignant tumors, 751, *757
Bacilli: See Bacteria
Bacteria: See also under Meningococci; Pneumococci; Staphylococci; Streptococci; Tubercle Bacilli; Typhoid, bacilli; Viruses; etc.
Abortus and Melitense Group: See also Undulant fever
abortus and melitense group; precipitable substance from genus Brucella, 285
Aertrycke: See Salmonella, aertrycke
antigenic properties combined with antibodies, 131
contamination of bacterial culture by organism resembling Hartmannella Castellani, 129
diffusion factor in culture filtrates, 130
Eberth's: See Typhoid, bacilli
effect of purified enzymes on viruses and gram-negative bacteria, 724
enterococci; Ludwig's angina due to esculin-fermenting hemolytic streptococcus (enterococcus), 295
fusiformis; immunologic and biochemical properties of proteins extracted from fusobacteria, anaerobic streptococci and Bacterium melaninogenicum, 741
lesions produced by bacteria and bacterial toxins, *103
mediums for Bacillus typhosus, 599
mycobacteria; dissociation of unusual acid-fast organisms, 891
Necrophorus: See Actinomycosis
Paratyphoid: See Salmonella
Pertussis: See Whooping cough
reaction of mice and mouse tumors to injections of bacterial products, 733
Shiga's: See Dysentery
Tularense: See Tularemia
Bacterium: See Bacteria
Baker, A. B.: Cerebral lesions in hypoglycemia, *190
Basophils: See Pituitary Body
Baths, simple small paraffin ovens or constant temperature baths constructed at small cost, *539
Bedouin, blood grouping of Rwala Bedouin, 131
Benzanthracene, blood changes in mice in sarcoma due to, 735
elimination of 1,2-benzpyrene, 1,2,5,6-dibenzanthracene and anthracene from blood stream, 903
morphology of sarcomas produced by 1,2,5,6-dibenzanthracene, 135

Benzanthracene—Continued
spindle cell tumors and leukemia in mice after injection of water-soluble compound of 1,2,5,6-dibenzanthracene, 901
studies in carcinogenesis; detection of dibenzanthracene in mouse tumors induced by hydrocarbons, 135
transplantation of tumors induced by 1,2,5,6-dibenzanthracene in albino mice, 901
Benzopyrene, elimination of 1,2-benzpyrene, 1,2,5,6-dibenzanthracene and anthracene from blood stream, 903
studies in carcinogenesis; compounds related to benzopyrene, 746
Bergstrom, V. W.: Recurrent sarcoma of uterus, 609
Berlock Dermatitis: See Pigmentation, cutaneous
Bernstein, M. A.: Classification of arthritis based on pathologic observations in joints, 752
Bighead in sheep, *422
Bile, attenuation of typhus virus by, 894
values of phosphatase in serum and bile of dogs with experimental biliary fistulas and in dogs with complete biliary obstruction, 739
Bile Ducts: See also Biliary Tract; Gallbladder
changes in liver of cat following ligation of single hepatic ducts, *641
foreign body of long standing in biliary ducts, 747
Biliary Tract: See also Bile Ducts; Gallbladder; Liver
effects of removal of functioning gallbladder, 580
values of phosphatase in serum and bile of dogs with experimental biliary fistulas and in dogs with complete biliary obstruction, 739
Bilirubin in Blood: See Blood, bilirubin
Biologic products, procedure and apparatus for preservation in lyophil form of serum and other biologic substances, 448
Biskind, G. R.: Studies in histochemistry; vitamin C in testes in relaton to anatomic and to functional changes, *363
Blood: See also Erythrocytes; Hemoglobin and Hemoglobin Compounds; Leukocytes
action of human and animal blood on meningococcus, 132
bactericidal properties of, 134
bilirubin content, and urobilinogen content of urine in diabetes mellitus, 720
coagulation; Weltmann serum test, 143
correlation of serum iron, bone marrow and blood cell changes following specific therapy in macrocytic anemias, 738
Diseases: See Anemia; Leukemia; etc.
fat deposition in arteries treated in vitro with B factor, *332
fibrin; effect of dissociation of streptococci on their fibrinolytic and anticlotting activity, 283
Groups: See also Agglutination; Blood transfusion
groups; abnormal iso-antibodies following transfusions, 440
groups; agglutinogens, 731
groups; agglutinogens M and N, 899
groups; agglutinogens M and N in allergic conditions, 898
groups; agglutinogens M and N of Ainu, 897
groups; blood grouping of Rwala Bedouin, 131
groups; determination on dried blood spots and traces of saliva, 597
groups; exclusion of maternity, 597
groups; hereditary defect of N agglutinogen, 291
groups; immune hemagglutinin in rabbit and new blood group factor, 134
groups; immunization of rabbits with human serums of different blood groups, 732
groups, in forensic medicine, 597
groups; influence of temperature on iso-agglutination, 598
of female mice (breeders) of cancer-susceptible (A) and cancer-resistant (CBA) strains, *202
Blood—Continued
Parasites: See Malaria
platelets, technical methods for study of; critical review with bibliography, *850
pressure, changes in appearance of wall of muscular artery between diastolic and systolic blood pressures, 884
pressure, high; benign and malignant hypertension and nephrosclerosis, 431
pressure, high; inflammatory lesions in glomeruli in pyelonephritis in relation to hypertension and renal insufficiency, 432
pressure, systolic, variations in patients with renal tumors, 734
production of fat granules and of degeneration in cultures of adult tissue by agents from blood plasma, *316
proteins; blood plasma protein regeneration controlled by diet, 123
proteins; serologic comparison of phosphoprotein of serum of laying hen and vitellin of egg yolk, 730
sternal marrow in pernicious anemia; correlation of observations at biopsy with blood picture and effects of specific treatment in megaloblastic ("liver-deficient") hyperplasia, *159
sugar; cerebral lesions in hypoglycemia, *190
sugar; chronic hepatogenic hypoglycemia due to ascending cholangiolitis with recovery following cholecystectomy, 739
transfusion, abnormal iso-antibodies following, 440
transfusion accident following repeated use of same blood donor, 898
tryptophan, clinical significance in infectious diseases, 900
urea; effect of roentgen rays on blood urea of rats immune to Jensen's sarcoma, *515
values of phosphatase in serum and bile of dogs with experimental biliary fistulas and in dogs with complete biliary obstruction, 739
Bogen, E.: Monilial meningitis, 909
Bollman, J. L.: Factors influencing fatty condition in dog's liver produced by alcohol and diet rich in fat, 740
Bones, Diseases: See Osteomyelitis; etc.
formation in lung and trachea, 435
fragility; endocrine system in osteogenesis imperfecta, 585
growth; osteogenesis in "marble bone" disease, 721
Marble: See Osteosclerosis
marrow, changes in agranulocytosis, 277
marrow; changes in outlying bone marrow accompanying local increase of temperature within physiologic limits, 882
marrow; correlation of serum iron, bone marrow and blood cell changes following specific therapy in macrocytic anemias, 738
marrow; experimental radium poisoning, 577
marrow in anemias as studied by sternal biopsy, 292
marrow in Brown-Pearce carcinomatosis of rabbit, 290
marrow; increase in reticulo-endothelial cells in outlying bone marrow consequent on local increase in temperature, 883
marrow; paralysis syndrome produced in rabbits and guinea-pigs by extracts of normal primate bone marrow, 578
marrow; sternal marrow in pernicious anemia; correlation of observations at biopsy with blood picture and effects of specific treatment in megaloblastic ("liver-deficient") hyperplasia, *159
primary lymphosarcoma of liver with metastases to marrow and secondary anemia, *674
Book Reviews:
Aids to Pathology; H. Campbell and K. Campbell, 911
Autopsy Diagnosis and Technique: Manual for Medical Students, Practitioners, Pathologists and Coroners' Physicians; O. Saphir, 613
British Masters of Medicine; edited by D. Power, 155

Book Reviews—Continued
Chondromalacia Patellae; A. Øwre, 910
Effet des rayons X sur les os longs en development et sur la formation de cal. Étude radiobiologique et anatomique chez la rat; B. Dahl, 910
Endocrinology in Modern Practice; W. Wolf, 155
Fundamentals of Electrocardiographic Interpretation; J. B. Carter, 754
Harvey Lectures, Delivered Under the Auspices of the Harvey Society of New York, 1935-1936, Under Patronage of New York Academy of Medicine; M. Bergmann and others, 157
Histological Technic; A. A. Krajian, 156
Hundred Years of Medicine; W. E. B. Lloyd, 456
Infection bacillaire et la tuberculose chez l'homme et chez les animaux. Etude biologique et expérimentale. Vaccination préventive; A. Calmette, 753
Juvenile Paresis; W. C. Menninger, 613
Medical Classics; compiled by E. C. Kelly, 156
Précis de parasitologie; E. Brumpt, 612
Report of Medical Research Council for Year 1935-1936, 755
Text-Book of Pathology; R. Muir, 157
Use of Developing Egg in Virus Research; F. M. Burnet, 754

Books received, 158, 298, 614, 756, 912
Bordet-Gengou Bacillus: See Whooping cough
Botryomycosis Hominis: See Granuloma pyogenicum
Bradford, F. K.: Dermoid cyst of spinal cord, 454
Brain: See also Cerebellum; Corpus Striatum; Meninges; Nervous System; etc.
abnormalities; arhinencephaly with extreme eversion of end-brain, 719
abscess, actinomycotic, *58
Aneurysm: See Aneurysm, cerebral
argentophil deposits in cancer, 127
atrophy; olivopontocerebellar atrophy, 433
carcinoma, papillomatous, in lateral ventricle, 447
cerebral lesions in hypoglycemia, *190
cortical encephalomalacia in infancy, 885
genesis of multiple encephalomalacia and of angina pectoris in foam formation, 280
gliomas, 595
is all colloid found in midbrain of hypophysial origin? 280
primary melanosarcoma, numerous pigmented nevi of skin and extensive neurofibromatosis of cutaneous nerves, 447
psittacosis; lesions of central nervous system, 439
reacting cells in brain in presence of foreign body, 885
relation of cerebrum to cerebellum, 579
ruptured cerebral varices, 124
serologic relations between brain and testicle, 443
tumors, multiple primary, 444
tumors, spinal fluid of patients with, 124
varicosis at base of, 888
Branchial apparatus, suprascapular branchial cyst, 889
Breast, adenocarcinoma, hormonal pathogenesis of, 900
cancer, associated with metastatic calcification, 748
cancer, effect of estrin on incidence in mice, 901
carcinoma, with condition of blood simulating chronic lymphatic leukemia, *531
inflammation; giant cells in chronic mastitis, 280
tumors, primary, of pituitary glands, ovaries and mammary glands in mouse, 288
Brenner Tumor: See Ovary, tumors
Bright's Disease: See Nephritis
Bronchi, cyst, congenital bronchogenic, of mediastinum in infant, 296
lung tissue culture, 581
Bronchiectasis and metaplasia in lung of rat, 584
experimental production in dogs, 606
Bronchogenic Cancer: See under Lungs, cancer
Bronchopneumonia and experimental pulmonary edema, 744
Brooks, C.: Parenteral protein in pneumonias, 744
Brown, J. W.: Anatomic and bacteriologic findings in infections with specific types of pneumococci, including types I to XXXII, *801
Brucella Abortus: See Bacteria, abortus and melitense group
Buckwheat poisoning, fagopyrism, *416
Burket, L. W.: Nasopalatine duct structures and peculiar bony pattern observed in anterior maxillary region, *793

Calcification: See also Bones, growth; etc.
metastatic, associated with carcinoma of breast, 748
Calcium metabolism in scleroderma, 607
Campbell, D. H.: Active immunization of albino rats with chemical fractions from cat tapeworm (Taenia taeniaformis) and its larval form (Cysticercus fasciolaris [Crassicollis]), 751
Cancer: See also Adenocarcinoma; Sarcoma; Tumors; etc.; and under names of organs and regions, as Brain, cancer; Breast, cancer; Liver, cancer; Lungs, cancer; etc.
argentophil deposits in brain in, 127
blood of female mice (breeders) of cancer-susceptible (A) and cancer-resistant (CBA) strains, *202
bone marrow in Brown-Pearce carcinomatosis of rabbit, 290
cells, macronucleolus in, 288
council, 713
detection of dibenzanthracene in mouse tumors induced by hydrocarbons, 135
influence of Reynals-McLean factor versus homologous tumor material on Brown-Pearce rabbit tumor, 741
of skin and xeroderma pigmentosum due to sunlight, *409
precancer; senile macular pigmentation, 721
production by hydrocarbons other than those of phenanthrene type, 593
production in rabbits with tobacco tar, 137
reaction of spontaneous tumors in mice to cystine di-sulfoxide, 444
rôle of hypophysis in rat, 288
seasonal influence in development of tar tumors in mice, 288
sensitivity of different sites of skin of mouse to carcinogenic agents, 594
serology of tumors, 596
studies in carcinogenesis; compounds related to benzopyrene, 746
studies in carcinogenesis; production of tumors in mice with hydrocarbons, 135
Cannon, P. R.: Experimental pulmonary edema and bronchopneumonia, 744
Inadequacy of allergic inflammation as protection against infection of rabbits with virulent pneumococci, 145
Cantarow, A.: Changes in liver of cat following ligation of single hepatic ducts, *641
Capillaries, intercapillary lesions in renal glomeruli, 432
Carbohydrates: See also Dextrose; Glycogen; etc.
antigenicity of, 287
carbohydrate-containing proteins of hemolytic streptococcus, 440
heterophile hapten and specific polysaccharide hapten of smooth form of Bacterium Shigae, 285
immunologic study of polysaccharide of typhoid bacillus, 441
Carbon monoxide, effects of exhaust gases from internal combustion engines and of tobacco smoke on mice, with reference to incidence of tumors of lung, 903
Carcinoma: See Cancer
Casey, A. E.: Influence of Reynals-McLean factor versus homologous tumor material on Brown-Pearce rabbit tumor, 741
Catalepsy, experimental, in cats, 881
Catron, L.: Nontransmissibility in utero of trichinosis in rat, 742

Cells: See also Cancer, cells; Phagocytes and Phagocytosis; Tissue; etc.
cytoplasmic inclusion bodies in human throat, 590
foreign body reaction of myocardium with reference to formation of giant cells and so-called myocytes, 743
giant, in chronic mastitis, 280
inclusions; salivary gland disease of mice, 283
inclusions; visceral lesions produced in mice by salivary gland virus of mice, 437
nuclear alterations following intravenous injections of glucose and of other solutions, 582
nuclei; karyoclastic poisons, 887
Cerebellum, olivopontocerebellar atrophy, 433
relation of cerebrum to, 579
Cerebrospinal Fluid of patients with tumor of brain, 124
serologic demonstration of causative agent in spinal fluid in cases of meningitis, 295
Chandler medal, 122
Chemistry, lesions produced by inorganic and organic chemical compounds, *73
Chemotropism: See Leukocytes
Chest: See Thorax
Chiari Network: See Heart, abnormalities
China and Chinese, serologic, toxigenic and biochemical reactions of hemolytic streptococci from throat of Hongkong Chinese, 893
Chlorine, effect of therapeutic inhalation on formation of antibodies, 899
Chloroform, new formation of reticulum fibers, 280
Chloroleukemia: See Chloroma
Chloroma, spontaneous leukemia and chloroleukemia in rat, 136
Chorio-epithelioma of uterus, 5 cases, 453
Choriomeningitis: See under Meningitis
Chorion, determination of duration of pregnancy by erythrocyte count in capillaries of chorionic villi, 126
Chromosomes, sensitivity to roentgen rays in relation to temperature and p_H, 745
Clark, J. H.: Influence of thymus extract on certain endocrine organs of rat, 293
Clover Disease: See Trifoliosis
Cobalt, karyoclastic poisons, 887
Colds: See Respiratory Tract, diseases
Colitis, chronic ulcerative, further studies on Bacterium necrophorum from, 453
production in dog with staphylococcus toxin, *634
ulcerative; streptococci from human gastrointestinal ulcerations and from bovine mastitis, 438
Colloids, localization and concentration of antibodies and colloidal dye in areas of inflammation of various ages, 145
Colon, melanosis of, 610
Compere, E. L.: Pathologic comparison of tuberculosis and pyogenic infection of spine, 454
Conn, J. W.: Chronic hepatogenic hypoglycemia due to ascending cholangiolitis with recovery following cholecystectomy, 739
Cor Pulmonale: See Heart, dilatation
Cornbleet, T.: Calcium metabolism in scleroderma, 607
Coronary Arteries: See under Arteries
Corpora Amylacea: See Amyloidosis
Corpus Striatum, unilateral traumatic selective degeneration of pallidum and striatum, 583
Coryza: See Respiratory Tract, diseases
Curtis, S. H.: Multiple myeloma; report of case, 609
Cushing Syndrome: See under Pituitary Body
Custer, R. P.: Bone marrow in anemias as studied by sternal biopsy, 292
Histogenesis of plasmocyte, 748
Cylindroma of palate, 447
Cystadenoma, uterine papillary, with origin in rests of wolffian body; report of case, 600
Cystine, reaction of spontaneous tumors in mice to cystine di-sulfoxide, 444
Cysts: See under names of organs and regions, as Bronchi; etc.
Dermoid: See Tumors, dermoid

Dack, G. M.: Further studies on Bacterium necrophorum from chronic ulcerative colitis, 453
Dameshek, W.: Sternal marrow in pernicious anemia; correlation of observations at biopsy with blood picture and effects of specific treatment in megaloblastic ("liver-deficient") hyperplasia, *159
d'Aunoy, R.: Experimental pathologic study of lymphogranuloma venereum, 745
Davidsohn, I.: So-called superior pulmonary sulcus tumor, 906
Davison, C.: Lesions of nervous system of rat in vitamin B deficiency, *207
Death, shock; mechanism of death following intestinal obstruction, 579
sudden, in case of schistosomiasis of lungs, 660
Degeneration, fatty; perirenal and peripelvic fibrolipomatosis, 434
production of fat granules and of degeneration in cultures of adult tissue by agents from blood plasma, *316
Delaney, P. A.: Modification of Ziehl-Neelsen method of staining tubercle bacilli and tissues in paraffin or pyroxylin (celloidin) sections; comparison of this method with Cooper's technic, *265
Dementia paralytica, specific infectiousness of syphilitic ganglions, 130
Dermatitis, Berlock: See Pigmentation, cutaneous
multiformis; virus of pemphigus and herpetiform dermatitis, 588
with secondary sensitivity to light, *415
Dermatomyositis, 719
Dermoid Cysts: See Tumors, dermoid
Dextrose, nuclear alterations following intravenous injections of glucose and of other solutions, 582
Diabetes Mellitus: See also Blood sugar
bilirubin content of blood and urobilinogen content of urine in, 720
chronic periarteritis nodosa, 435
effects of adrenalectomy and hypophysectomy on experimental diabetes in cat, 276
Dibenzanthracene: See Benzanthracene
Dick Test: See under Scarlet fever
Digestive Tract: See also Gastro-Intestinal Tract; Intestines; Stomach; etc.
neurogenous tumors of, 904
Diphtheria, immunity; immunization with mixtures of diphtheria anatoxin (Ramon) with aluminum hydroxide, 286
normal antitoxin, 287
one dose immunization against diphtheria with precipitated anatoxin, 134
Dixon, H. M.: Chemotropism in leukocytes; source of attractive substance, 743
Doan, C. A.: Correlation of serum iron, bone marrow and blood cell changes following specific therapy in macrocytic anemias, 738
Donors: See Blood transfusion
Dragstedt, L. R.: Further studies on Bacterium necrophorum from chronic ulcerative colitis, 453
Drugs, thymus and adrenals in response of organism to injuries and intoxications, 580
Dyes: See Stains and Staining
Dysentery, heterophile hapten and specific polysaccharide hapten of smooth form of Bacterium Shigae, 285
Dysgerminoma, ovarian tumors (granulosa cell carcinoma, arrhenoblastoma, dysgerminoma, Brenner tumor), 288

Ectromelia, Infectious, propagation of virus of mice in developing egg, 726
virus of, 594
Edema: See Elephantiasis; Lungs, edema; Spleen, edema; etc.
Eggs, serologic comparison of phosphoprotein of serum of laying hen and vitellin of egg yolk, 736
Egoville, J. W.: Metastatic calcification associated with carcinoma of breast, 748
Elephantiasis, lymphedema of extremities from invasion of lymphatic vessels by cancer cells, 902

Embolism: See also Thrombosis
certain effects of pulmonary gas embolism, 716
genesis of multiple encephalomalacia and of angina pectoris in foam formation, 280
paradox of Chiari's network, 718
Encephalitis: See also Encephalomyelitis
effect of reticulo-endothelial blockade on vaccination, 592
epizootic, in foxes, 586
experimental intranasal infection with certain neurotropic viruses; prevention by means of chemicals instilled into nostrils, 439
experimental (St. Louis type) in mice with high inborn resistance, 724
hemorrhagic, following treatment with sulpharsphenamine, death of 2 syphilitic infants, 431
Encephalomyelitis, equine, active immunization of guinea-pigs with formaldehydized virus of, 896
equine, neutralization of virus by immune serum, 896
Endocarditis, acute, produced by Bacillus paratyphosus B, *270
endocardial reaction of mitral and tricuspid valves of infants and children, 436
pathologic-anatomic study of, 721
subacute bacterial, streptococci of, 591
Endocrine Glands: See also Hormones
endocrine system in osteogenesis imperfecta, 585
influence of thymus extract on certain endocrine organs of rat, 293
Endometrium, histological studies during various phases of menstrual cycle, 278
Endothelioma of pleura, 904
so-called pleural endothelioma, 737
Endothelium: See Reticulo-Endothelial System
Endotoxin: See Salmonella, aertrycke
Enteritis: See under Intestines
Enterococcus: See Bacteria, enterococci
Enzymes, blood group ferment in saliva, 592
content of parenchymatous adenocarcinoma of pancreas, 135
purified, effect on viruses and gram-negative bacteria, 724
Epididymis, tumors of spermatic cord, epididymis and testicular tunics, 136
Epilepsy, corpora amylacea in nerve cells, 720
Epithelioma, intracutaneous, of rabbits and its immunizing effect, 446
Epithelium, epithelial metaplasia of thyroid gland with reference to histogenesis of squamous cell carcinoma of thyroid gland, *821
Ergosterol, Irradiated: See Viosterol
Erythroblastosis: See Hematopoietic System
Erythrocytes: See also Anemia; Hemoglobin and Hemoglobin Compounds; Hemolysis; etc.
detection of antigenic differences in mouse erythrocytes by means of immune serums, 441
determination of duration of pregnancy by erythrocyte count in capillaries of chorionic villi, 126
sternal marrow in pernicious anemia; correlation of observations at biopsy with blood picture and effects of specific treatment in megaloblastic ("liver-deficient") hyperplasia, *159
Escudero, L.: Experimental production of bronchiectasis in dogs, 606
Esophagus, diverticula, 888
primary lymphogranulomatosis of, 887
Estrin: See Hormones, sex
Estrogenic Substances: See Hormones, sex
Extremities, arteriographic study of collateral circulation, 433

Fagopyrism: See under Buckwheat poisoning
Fallopian Tubes, actinomycosis of tubes and ovaries, *53
lipoid cell granuloma after uterosalpingography, 888
Fasting, blood plasma protein regeneration controlled by diet, 123
Fat: See also Lipoids
comment on Kawamura-Yasaki fat staining method and modification in preparation of stock solution, 139
deposition in arteries treated in vitro with B factor, *332
morphology in tumors, 447
origin of fat and lipoids in tissue cultures, 717
production of fat granules and of degeneration in cultures of adult tissue by agents from blood plasma, *316
staining, new method, 138
Feldman, L.: So-called superior pulmonary sulcus tumor, 906
Fellowship, Mary Putnam Jacobi fellowship, 275
Femur, Ewing's sarcoma, 446
Ferments: See Enzymes
Fever: See also Malaria; Temperature; Typhoid; Typhus; etc.
acute postoperative necrosis of liver, 714
Artificial: See under Rabies
Undulant: See Undulant fever
Fibrin, experimental studies on primary changes during formation of thrombi, *307
Fibrinolysis: See Blood, fibrin
Fibrolipomatosis, perirenal and peripelvic, 434
Fibroma, change in rabbit fibroma virus suggesting mutation, 128
Filariasis in Puerto Rico, 283
Fingers and Toes, mixed tumor of salivary gland type involving finger, 447
Finland, M.: Anatomic and bacteriologic findings in infections with specific types of pneumococci, including types I to XXXII, *801
Fischer, M. H.: William Buchanan Wherry, 148
Fistula, values of phosphatase in serum and bile of dogs with experimental biliary fistulas and in dogs with complete biliary obstruction, 739
Fite, G. L.: Rare malformation of liver, 607
Flock, E.: Factors influencing fatty condition in dog's liver produced by alcohol and diet rich in fat, 740
Foot, N. C.: Criticism of laboratory routine in modern institutions of pathology, 749
Foot and Mouth Disease, purified virus of, 726
virus as influenced by tissue culture, 894
Foramen incisivum, nasopalatine duct structures and peculiar bony pattern observed in anterior maxillary region, *793
Foreign Bodies, encapsulation of foreign material in peritoneal cavity, 718
meningeal mesenchymoma of foreign body origin, 447
of long standing in biliary ducts, 747
reacting cells in brain in presence of, 885
Fox, H.: Chronic arthritis in lower mammals, 293
Fox, J. P.: Localization and concentration of antibodies and colloidal dye in areas of inflammation of various ages, 145
Frambesia, reinoculation with spirochetes of yaws, 728
Francis, L. D.: Blood of female mice (breeders) of cancer-susceptible (A) and cancer-resistant (CBA) strains, *202
Frontal Bone, Morgagni's syndrome—internal frontal hyperostosis, virilism and obesity, 723
Fungi, preparation of permanent gross colonies of fungi for teaching purposes, *681
Fusobacteria: See Bacteria, fusiformis

Gallbladder: See also Bile; Bile Ducts; Biliary Tract
function, studies of, 715
functioning, effects of removal of, 580
pleomorphic cell sarcoma of, 147
Ganglioglioneuroma of spinal cord, 144
Ganglions, changes in spinal cord and posterior root ganglions in sepsis, 722
Gardner, L. U.: Silicosis and related conditions (annual Gross lecture), 905

Gastro-Intestinal Tract: See also Colon; Digestive Tract; Intestines; Stomach; etc.
tetanus toxin in digestive tract, 286
Geeldikkop: See Tribulosis
Genitals, histologic changes in sex organs of spayed rats induced by testosterone and estrone, 716
Glick, D.: Studies in histochemistry; vitamin C in testes in relation to anatomic and to functional changes, *363
Glioma, 595
gliomatosis of leptomeninges, 902
Globin: See Hemoglobin and Hemoglobin Compounds
Globus Pallidus: See under Lenticular Nucleus
Glomerulonephritis: See Nephritis, glomerular
Glomus, glomal tumors, *615
Glucose: See Dextrose
Glycogen, cardiomegalia glycogenica circumscripta, 431
disease, 584
Gold, demonstration in animal tissues by method of physical development (germ method of Liesegang), 599
Gonadotropic Substances: See Hormones, sex
Gonads: See Endocrine Glands; Genitals; Ovary; Testicles
Gordon Test: See Lymphogranuloma
Gough, J. A.: Five cases of chorio-epithelioma of uterus, 453
Granuloma inguinale, specific histology of, *224
pyogenicum; botryomycosis in man, 602
Grattan, J. F.: Values of phosphatase in serum and bile of dogs with experimental biliary fistulas and in dogs with complete biliary obstruction, 739
Greenblatt, R. B.: Specific histology of granuloma inguinale, *224
Grethmann, W.: Pathogenesis of tuberculosis as general and systemic infection with reference to pulmonary tuberculosis, 451
Gross, H.: Sclerosis of hepatic veins in chronic congestive heart failure, *457
Gross, L.: So-called congenital bicuspid aortic valve, *350
Gross, P.: Hydraulic automatic tissue changer or immersion device, *392
Simple small paraffin ovens or constant-temperature baths constructed at small cost, *539
Growth curves of malignant and normal tissues of rat, 734
influence of thymus extract on certain endocrine organs of rat, 293

von Haam, E.: Experimental pathologic study of lymphogranuloma venereum, 745
Haemophilis Influenzae: See Influenza
Haptens: See Antigens and Antibodies
Hartley, G., Jr.: Inadequacy of allergic inflammation as protection against infection of rabbits with virulent pneumococci, 145
Hartmannella Castellanii: See Amebae
Hartsuch, P. J.: Chemical reaction between oleic acid and aqueous solutions of magnesium, 752
Hawkins, E. L.: Tuberculosis of tonsils, 909
Head, injuries; unilateral traumatic selective degeneration of pallidum and striatum, 583
Heart: See also Endocarditis; Myocarditis; etc.
abnormalities; mitral stenosis with interauricular insufficiency, 719
abnormalities; paradox of Chiari's network, 718
aneurysm of sinus of Valsalva with rupture into right auricle, *679
congenital syphilis with unusual histologic changes in myocardium, 2 cases, 450
dilatation; sickle-cell anemia; new cause of cor pulmonale, 277
displacement; transposition of arterial trunks, 146
effect of acute scurvy on guinea-pig heart, *20
experimental coronary occlusion, 577
factors influencing deposition of pigment in heart muscle, 888
Heart—Continued
foreign body reaction of myocardium with reference to formation of giant cells and so-called myocytes, 743
hypertrophy; cardiomegalia glycogenica circumscripta, 431
lesions in auriculoventricular conduction system in rheumatic fever, 277
morphologic changes in experimental myxedema, 578
perforation of intraventricular septum, 721
Rheumatic Disturbances: See under Rheumatic Fever
sclerosis of hepatic veins in chronic congestive heart failure, *457
Heinz, T. E.: Further studies on Bacterium necrophorum from chronic ulcerative colitis, 453
Hemagglutinins: See Blood, groups
Hematoma: See Meninges, hemorrhage
Hematopoietic System: See also Erythrocytes
connection between erythroblastosis (hemocytoblastosis), myelosis and sarcoma in chickens, 290
leuko-erythroblastic anemia, 886
Hemken, L.: Allergic lesions produced by injection of protein, 907
Hemochromogen: See Hemoglobin and Hemoglobin Compounds
Hemoglobin and Hemoglobin Compounds: See also Anemia; Blood; Erythrocytes; Hemolysis
pneumonoconiosis; iron pigment in anthracosis, 281
researches on carbon monoxide hemochromogens, 598
Hemolysis, changes of hemolytic titer after reinjections of antigens, 287
Hemophilis Pertussis: See Whooping cough
Hemsath, F. A.: Histology of homoplastic adrenal transplant that survived for 8 months in patient with Addison's disease, 141
Henthorne, J. C.: Rôle of anaerobic streptococci in human infections, *230
Hepatic Duct: See Bile Ducts
Hermaphroditism, topographic relations of prostate in, 586
Herpes virus, homogenized, properties of, 438
virus, virulence of, 125
Hirsch, E. F.: Primary lymphosarcoma of liver with metastases to marrow and secondary anemia, *674
Histamine, experimental intoxication and inflammation, 884
Hodgkin's Disease: See Lymphogranuloma
Horack, H. M.: Effect of artificial fever on experimental rabies, 742
Hormones: See also Endocrine Glands; and under names of organs, as Testicles; etc.
prolonged application of follicular hormone on rabbit uterus, 714
sex; changes induced by estrogens in adrenals of male mice, 581
sex; effect of estrin on incidence of mammary cancer in mice, 901
sex; gonadotropic hormone in testicular tumors, 448
sex; histologic changes in sex organs of spayed rats induced by testosterone and estrone, 716
sex; hormonal pathogenesis of adenocarcinoma of breast, 900
sex; metaplasia of uterine epithelium in rats after administration of estrin, 577
Horn, H.: Experimental nephropathies, *71, *241
Howe, J. S.: Rôle of anoxia of tissues in reaction to tuberculin, 740
Variability of tuberculin reaction, 908
Hydroa vacciniforme seu aestivale with porphyrinuria, caused by sunlight, *404
Hydrocarbons, experimental production of visceral tumors with, 593
production of cancer by hydrocarbons other than those of phenanthrene type, 593
studies in carcinogenesis; production of tumors in mice with hydrocarbons, 135

Hydrogen Ion Concentration, colony morphology of tubercle bacilli, 128
sensitivity of chromosomes to roentgen rays in relation to temperature and p_H, 745
Hydronephrosis, experimental contributions to study of, 883
in supernumerary kidney, 907
Hydrophobia: See Rabies
Hyperergy: See Anaphylaxis and Allergy
Hypericism, *418
Hypersensitiveness: See Anaphylaxis and Allergy
Hypertension: See Blood pressure, high
Hypoglycemia: See Blood sugar
Hypophysectomy: See under Pituitary Body
Hypophysis: See Pituitary Body
Hysterectomy: See under Uterus

Ikeda, K.: Lipoid pneumonia of adult type (paraffinoma of lung); report of 5 cases, *470
Immunity: See also Anaphylaxis and Allergy; Antigens and Antibodies; Diphtheria, immunity; Serum, immune; Streptococci; etc.
active immunization of albino rats with chemical fractions from cat tapeworm (Taenia taeniaformis) and its larval form (Cysticercus fasciolaris [Crassicollis]), 751
antigenic properties of bacteria combined with antibodies, 131
antigenic properties of hormones, 731
changes of hemolytic titer after reinjections of antigens, 287
immunization of rabbits with human serums of different blood groups, 732
mass factor in immunologic studies on viruses, 896
nonspecific allergy, 731
phenomenon of local skin reactivity to bacterial filtrates, 891
rôle of vitamin C in resistance, *543, *683
serologic relations between brain and testicle, 443
serologic specificity of tuberculous caseous matter, 443
synergy of antibodies, 442
toxic substances in urine and sweat in typhoid fever, 284
Infection, blood plasma protein regeneration controlled by diet, 123
clinical significance of tryptophan in blood serum in infectious diseases, 900
influence of infection and intoxication on hemoglobin production in anemia, 580
Inflammation, allergic, inadequacy as protection against infection of rabbits with virulent pneumococci, 145
and experimental histamine intoxication, 884
localization and concentration of antibodies and colloidal dye in areas of inflammation of various ages, 145
Influenza, acute degenerative myocarditis in, 722
epidemic, propagation of virus on developing egg, 130
human virus in artificial medium, 723
neutralization tests with serum of convalescent or immunized animals and viruses of swine and human influenza, 729
neutralizing antibodies for virus of human influenza in serums of persons of different ages, 729
neutralizing antibodies for virus of swine influenza in serums of human beings of different ages, 729
recovery of filtrable virus from children with influenza, 586
recovery of virus suspended in air and its destruction by ultraviolet radiation, 587
virus, propagation on developing egg, 892
Instruments: See Apparatus
Internal Secretions: See Endocrine Glands
Intestines: See also Colon; Gastro-Intestinal Tract
intestinal flora of chickens affected with enteritis and intestinal parasitism, 589
obstruction in infant due to unusual cause, 721
rôle of anaerobic streptococci in human infections, *230
Intestines—Continued
shock; mechanism of death following intestinal obstruction, 579
Intranuclear Inclusions: See Cells
Iodine, lipoid cell granuloma of fallopian tubes after uterosalpingography, 888
Iris, postmortem discoloration of, 598
Irradiation: See Radiations; Radium; Roentgen Rays; and under names of diseases and organs
Isaacs, R.: Stages in development of monocyte, 747
Iso-Agglutination: See Agglutination; Blood, groups
Iso-Agglutinins: See under Agglutinins; Blood, groups

Jacobi, Mary Putnam Jacobi fellowship, 275
Jaffé, R. H.: Epithelial metaplasia of thyroid gland with reference to histogenesis of squamous cell carcinoma of thyroid gland, *821
Jaws, nasopalatine duct structures and peculiar bony pattern observed in anterior maxillary region, *793
Joints, classification of arthritis based on pathologic observations in joints, 752
rôle of reticulo-endothelial system in deposition of colloidal and particulate matter in articular cavities, 579
Jones, T. D.: Effect of acute scurvy on guinea-pig heart, *20
Jordan, H. E.: Hemoblastic leukemia; study of case, *653
Jurisprudence, Medical; blood grouping in forensic medicine, 597
central nervous system in acute metallic poisoning, 291
forensic medicine, 576
medicolegal conference, 576

Kahn Test: See under Syphilis
Kaposi's Sarcoma: See Sarcoma
Karyoclasis: See Cells, nuclei
Kellert, E.: Melanosis of colon, 610
Kelman, H.: Tumor of superior thoracic inlet (tumor of superior pulmonary sulcus); clinicopathologic study, *383
Kessel, J.: Monilial meningitis, 909
Kidneys, antigenic function of, 591
bilateral cortical necrosis in pregnancy, 434
denervated, hyperergic reaction of, 581
denervation, lesions produced by, *241
Diseases: See also Hydronephrosis; Nephritis; Pyelonephritis
diseases; experimental nephropathies, *71, *241
glycogen disease, 584
hydronephrosis in supernumerary kidney, 907
inflammatory lesions in glomeruli in pyelonephritis in relation to hypertension and renal insufficiency, 432
lesions produced by bacteria and bacterial toxins, *103
lesions produced by excessive ingestion of protein or irradiated food products, *95
lesions produced by inorganic and organic chemical compounds, *73
lesions produced by irradiation, *91
lesions produced by parenteral injections of proteins and nephrotoxins, *245
perirenal and peripelvic fibrolipomatosis, 434
tuberculosis, 143
tumors; variations in systolic blood pressure in patients with renal tumors, 734
waste pigment of renal epithelium, 889
weight of normal adult human kidneys and its variability, *493
King, L. S.: Connective tissue reaction in multiple and in diffuse sclerosis, *338
Klein, R. I.: Weltmann serum test, 143
Klinck, G. H., Jr.: Lipoid pneumonia; report of case, 608
Krafka, J., Jr.: Mechanical factors in arteriosclerosis, *1

Laboratories, criticism of laboratory routine in modern institutions of pathology, 749
Larson, C. P.: Museum mountings at minimum cost, *536

Larynx, immunologic studies of virus of infectious laryngotracheitis of fowls, with "developing egg technic," 895
infraglottic perforating tuberculous ulcer of, 432
Latitude, antistreptolysin titer in relation to, 132
Laufman, H.: Hydronephrosis in supernumerary kidney, 907
Lebowich, R. J.: Mediastinal teratoma with secondary squamous cell carcinoma, 608
Lens, Crystalline; studies in synergy, 895
Lenticular Nucleus, unilateral traumatic selective degeneration of pallidum and striatum, 583
Leptomeninges: See Meninges
Leukemia and spindle cell tumors in mice after injection of water-soluble compound of 1, 2, 5, 6-dibenzanthracene, 901
carcinoma of breast with condition of blood simulating chronic lymphatic leukemia, *531
hemoblastic, study of case, *653
leukosis and sarcomatosis of chickens; unity of virus, 137
monocytic, report of case, 609
myelogenous; connection between erythroblastosis (hemocytoblastosis), myelosis and sarcoma in chickens, 290
spontaneous, and chloroleukemia in rat, 136
Leukocytes: See also Leukemia; Mononucleosis; Phagocytes and Phagocytosis; etc.
blood changes in mice in sarcoma, 735
chemotropism in; source of attractive substance, 743
stages in development of monocyte, 747
transfers of living leukocytes, 743
Leukosis: See Leukemia
Lev, M.: Transposition of arterial trunks, 146
Levin, E. J.: Museum mountings at minimum cost, *536
Levine, J.: Actinomycosis of tubes and ovaries, *53
Levinson, S. A.: Weltmann serum test, 143
Lichtenstein, B. W.: Actinomycotic abscess of brain, *58
Ganglioglioneuroma of spinal cord, 144
Lieberthal, F.: Renal tuberculosis, 143
Light: See also Radiations; Ultraviolet Rays
dermatitis with secondary sensitivity to, *415
malignant tumors from solar irradiation, 290
photosensitization and photodynamic diseases of man and lower animals, *399
Lilly award, 880
Lipids: See Lipoids
Lipogranulomatosis, progressive lipomatosis of musculature with lipocalcinogranulomatosis, 722
Lipoids: See also Fat; etc.
changes of hemolytic titer after reinjections of antigens, 287
fixed antigens of tubercle bacillus, 133
lipoid pneumonia of adult type (paraffinoma of lung); report of 5 cases, *470
lipoid pneumonia; report of case, 608
waste pigment of renal epithelium, 889
Lipoma of brain and spinal cord, 596
Lisa, J. R.: Actinomycosis of tubes and ovaries, *53
Two cases of congenital syphilis with unusual histologic changes in myocardium, 450
Unusual findings in acute coronary thrombosis, 449
Liver: See also Biliary Tract
abnormalities; rare malformation of, 607
acute postoperative necrosis of, 714
arterial circulation in normal human liver, 436
atrophy, massive acute, 887
carcinoma, experimental, 596
changes in cat following ligation of single hepatic ducts, *641
chronic hepatogenic hypoglycemia due to ascending cholangiolitis with recovery following cholecystectomy, 739
cirrhosis, architecture of small nodule form, 281
cirrhosis, modern concepts of, 142
Liver—Continued
diseases; bilirubin content of blood and urobilinogen content of urine in diabetes mellitus, 720
factors influencing fatty condition in dog's liver produced by alcohol and diet rich in fat, 740
glycogen disease, 584
primary lymphosarcoma of liver with metastases to marrow and secondary anemia, *674
size of spleen and liver-spleen ratio; statistical study based on 1,000 autopsies, *33
Louping-Ill: See Encephalomyelitis
Lucké, B.: Tumor in tadpole, 292
Lufkin, N. H.: Cerebral lesions in hypoglycemia, *190
Lungs: See also Pleura; Respiratory Tract; etc.
abscess; immunologic and biochemical properties of proteins extracted from fusobacteria, anaerobic streptococci and Bacterium melaninogenicum, 741
abscess, morbid histology of walls of, 434
bone formation in lung and trachea, 435
cancer, and pulmonary asbestosis, 734
cancer; bronchiectasis and metaplasia in lung of rat, 584
cancer; bronchogenic carcinoma, 136
cancer; pathologic changes in primary cancer of lung, 138
cancer, primary, 445
cancer; primary diffuse alveolar carcinoma of lung, 595
cancer, squamous cell, in asbestosis, 446
edema, experimental, and bronchopneumonia, 744
fate of iodized poppy-seed oil 40 per cent in pulmonary parenchyma in man, 887
lipoid pneumonia of adult type (paraffinoma of lung); report of 5 cases, *470
lung tissue culture, 581
reaction of lung of rabbit in acute anaphylactic shock, 899
relations between inflammatory processes of lung and reactions of respective lymph glands, 126
rôle of anaerobic streptococci in human infections, *230
schistosomiasis of, sudden death in case of, 600
so-called superior pulmonary sulcus tumor, 906
Tuberculosis: See under Tuberculosis
tumors, effects of exhaust gases from internal combustion engines and of tobacco smoke on mice with reference to incidence of, 903
Lymph, dust transport and lymph circulation in lung, 281
Lymph Nodes: See also Lymphogranuloma; Lymphosarcoma; etc.
course of vital storage in, 717
experimental radium poisoning, 577
relations between inflammatory processes of lung and reactions of respective lymph glands, 126
specific infectiousness of syphilitic ganglions, 130
time of appearance of spirochetes of syphilis in regional lymph nodes, 727
variations in pathogenicity of spirochete of syphilis, 893
Lymphangioma, cystic, of adrenal gland, 137
Lymphatic System: See also Lymph Nodes
lymphedema of extremities from invasion of lymphatic vessels by cancer cells, 902
Lymphedema: See Elephantiasis
Lymphocytes in Meningitis: See Meningitis
Lympho-epithelioma and reticulosarcoma, 138
Lymphogranuloma: See also Granuloma
Gordon test for Hodgkin's disease, 587
Hodgkin's disease of stomach with fatal gastric hemorrhage, 140, *844
primary lymphogranulomatosis of esophagus, 887
venereum, cultivation of virus in mice, 588
venereum, experimental pathologic study of, 745
Lymphosarcoma, primary, of liver with metastases to marrow and secondary anemia, *674

McBroom, J.: Effect of acute scurvy on guinea-pig heart, *20
McCrea, F. D.: Effect of artificial fever on experimental rabies, 742
McCutcheon, M.: Chemotropism in leukocytes; source of attractive substance, 743
McDonald, J. R.: Rôle of anaerobic streptococci in human infections, *230
Maddock, S.: Values of phosphatase in serum and bile of dogs with experimental biliary fistulas and in dogs with complete biliary obstruction, 739
Magnesium, chemical reaction between oleic acid and aqueous solutions of magnesium, 752
Malaria, choroid melanin and serum flocculation in, 133
Malformations: See Abnormalities and Deformities; and under names of organs and regions
Malta Fever: See Undulant fever
Mammary Gland: See Breast
Mandible: See Jaws
Mann, F. C.: Factors influencing fatty condition in dog's liver produced by alcohol and diet rich in fat, 740
Marble Bones: See Osteosclerosis
Marmorston, J.: Rôle of vitamin C in resistance, *543, *683
Marshak, A.: Sensitivity of chromosomes to roentgen rays in relation to temperature and *p*H, 745
Massopust, L. C.: Infra-red photography of gross anatomic specimens, *67
Mastitis: See Breast, inflammation; Udders, inflammation
Maternity, exclusion of, 597
Mathews, F. P.: Photosensitization and photodynamic diseases of man and lower animals, *399
Maxillary Bone: See Jaws
Medal, Chandler medal, 122
Walter Reed medal, 122
Mediastinum, congenital bronchogenic cyst in infant, 296
mediastinal teratoma with secondary squamous cell carcinoma, 608
Mediums: See also under names of bacteria, i. e., Tubercle Bacilli; etc.
for Bacillus typhosus, 599
Megaloblast: See Erythrocytes
Melanin, pigmentation; melanosis of colon, 610
Melanoma, 900
Melanosarcoma, primary, of brain, numerous pigmented nevi of skin and extensive neurofibromatosis of cutaneous nerves, 447
Melanosis: See Melanin, pigmentation
Melnick, P. J.: Time factor in irradiation of malignant tumors, 751, *757
Meninges, gliomatosis of leptomeninges, 902
hemorrhage; surgical pathology of subdural hematoma, 433
meningeal mesenchymoma of foreign body origin, 447
Meningitis: See also Meningococci
epidemic in mouse colony due to virus of acute lymphocytic choriomeningitis, 590
epidemiology of lymphocytic choriomeningitis in white mice, 725
monilial, 909
rôle of anaerobic streptococci in human infections, *230
serologic demonstration of causative agent in spinal fluid in, 295
tuberculous, pathogenesis of, 889
Meningococci: See also under Meningitis
action of human and animal blood on, 132
standardization and concentration of antimeningococcus horse serum (type I), 131
Meningo-Encephalitis: See Encephalitis; Meningitis
Menstruation, histological studies of endometrium during various phases of menstrual cycle, 278
Mesenchymoma, meningeal, of foreign body origin, 447
Mesonephroma ovarii, 603
Mesonephros: See Wolffian Body
Metals, Poisoning: See Poisons and Poisoning
Midbrain: See Brain
Milk, irradiated, in experimental inhalation tuberculosis in guinea-pigs, 890
Mitral Valve, endocardial reaction of mitral and tricuspid valves of infants and children, 436
mitral stenosis with interauricular insufficiency, 719
Monilia; monilial meningitis, 909
Monocytes: See Leukemia, monocytic; Leukocytes
Mononucleosis, infectious, 133
Moore, C. V.: Correlation of serum iron, bone marrow and blood cell changes following specific therapy in macrocytic anemias, 738
Moore, R. A.: Pathology of prostatic utricle, *517
Morgagni's Syndrome: See under Frontal Bone
Morgan, D. R.: Changes in liver of cat following ligation of single hepatic ducts, *641
Mote, J. R.: Effect of acute scurvy on guinea-pig heart, *20
Mouth, buccal leukoplakia, 734
Mucin: See under Stomach
Müller's Duct, pathology of prostatic utricle, *517
Mumps: See Parotitis
Muscles, progressive lipomatosis of musculature with lipocalcinogranulomatosis, 722
Museum, clinicopathologic, 275
mountings at minimum cost, *536
Myasthenia gravis, thymoma and thymic hyperplasia in, 718
Mycobacteria: See Bacteria, mycobacteria
Myeloma, multiple, report of case, 609
Myelopoiesis: See Bones, marrow
Myelosis: See Leukemia, myelogenous
Myocarditis, acute degenerative, in influenza, 722
Myocardium: See under Heart
Myxedema, experimental, morphologic changes in heart in, 578

Nasopalatine Canal: See Foramen incisivum
Neck, technic for removal of organs of neck, 599
Necropsies: See Autopsies
Necrosis, collagen and reticulum fibers in necrotic foci, 584
Neely, J. M.: Sarcoma of uterus, *525
Negri Bodies: See Rabies
Nelson, P. M.: Antibodies for Trichomonas foetus, 744
Neoplasms: See Tumors
Nephritis: See also Kidneys, diseases; Pyelonephritis
glomerular; intercapillary lesions in renal glomeruli, 432
glomerular; lesions produced by parenteral injections of proteins and nephrotoxins, *245
Nephropathy: See under Kidneys
Nephrosclerosis: See Arteriosclerosis, renal
Nephrosis: See Kidneys, diseases; Nephritis
Nephrotoxins: See Nephritis, glomerular
Nerves, Cells: See Neurons
olfactory; olfactory bulbs in experimental poliomyelitis, 589
regeneration of posterior root fibers in cat, 881
Tumors: See under names of tumors
Nervous System: See also Brain; Cerebellum; Nerves; Spinal Cord; etc.
central, in acute metallic poisoning, 291
cerebral lesions in hypoglycemia, *190
effect of nerve stimulation on hyperergic inflammatory reaction, 123
lesions in rat in vitamin B deficiency, *207
lesions of central nervous system in trypanosomiasis, 125
lipomas of brain and spinal cord, 596
neurogenous tumors of digestive tract, 904
Neter, E.: Case of Ludwig's angina due to esculin-fermenting hemolytic streptococcus (enterococcus), 295
Serologic demonstration of causative agent in spinal fluid in cases of meningitis, 295
Neurofibromatosis, primary melanosarcoma of brain, numerous pigmented nevi of skin and extensive neurofibromatosis of cutaneous nerves, 447
Neurohypophysis: See Pituitary Body
Neurons, corpora amylacea in nerve cells, 720

Neurotropism: See Encephalitis
Nevi, primary melanosarcoma of brain, numerous pigmented nevi of skin and extensive neurofibromatosis of cutaneous nerves, 447
transformation of nevus cells into sebaceous gland cells? 279
Nickel, karyoclastic poisons, 887
Nicotine, experimental chronic poisoning, 276
Nose, survival of virus of poliomyelitis in oral and nasal secretions of convalescents, 724

Obesity, Morgagni's syndrome—internal frontal hyperostosis, virilism and obesity, 723

OBITUARIES:

Wherry, William Buchanan, 148

Old age, senile macular pigmentation, 721
Olfactory Bulb: See Nerves, olfactory
Opsonins, artificial opsonization of bacteria, 898
Osmosis and Permeability, diffusion factor in culture filtrates, 130
Osteogenesis: See Bones, growth
Imperfecta: See Bones, fragility
Osteomyelitis, pathologic comparison of tuberculosis and pyogenic infection of spine, 454
Osteosclerosis, osteogenesis in "marble bone" disease, 721
Ovary, actinomycosis of tubes and ovaries, *53
effect of hysterectomy on ovary of Macacus rhesus, 276
excision; histologic changes in sex organs of spayed rats induced by testosterone and estrone, 716
mesonephroma ovarii, 603
primary tumors of pituitary glands, ovaries and mammary glands in mouse, 288
some effects of ovariectomy during period of declining reproductive powers in mice, 715
tumors; granulosa cell carcinoma, arrhenoblastoma, dysgerminoma, Brenner tumor, 288
Ovens, simple small paraffin ovens or constant temperature baths constructed at small cost, *539
Oxidation reduction, rôle of anoxia of tissues in reaction to tuberculin, 740

Palate, cylindroma of, 447
mixed tumors of, 136
Pancreas, enzyme content of parenchymatous adenocarcinoma of, 135
Pancreatitis, acute hemorrhagic, pathogenesis of, 582
Papilloma, papilliomatous carcinoma in lateral ventricle, 447
Paraffin, simple small paraffin ovens or constant temperature baths constructed at small cost, *539
Paraffinoma, lipoid pneumonia of adult type (paraffinoma of lung); report of 5 cases, *470
Paralysis: See also Poliomyelitis
cortical encephalomalacia in infancy, 885
General: See Dementia paralytica
syndrome produced in rabbits and guinea-pigs by extracts of normal primate bone marrow, 578
Parasites: See under Intestines
Parotitis, histology of experimental mumps in monkey, 578
Paternity: See Blood, groups
Paul-Bunnell Test: See Mononucleosis, infectious
Pellagra due to sunlight, *411
Pemphigus, virus of pemphigus and herpetiform dermatitis, 588
Pepsin, commercial, group-specific A substance in, 896
Peptic Ulcers, pathogenesis of experimental ulcer, 717
Periarteritis nodosa, chronic, 435
Peritoneum, encapsulation of foreign material in peritoneal cavity, 718
pseudomyxomatous epithelial growth, 736
Perla, D.: Rôle of adrenal cortex in production and utilization of vitamin C; its influence on structure of teeth in albino rat, *372
Rôle of vitamin C in resistance, *543, *683
Perry, I. H.: Effect of roentgen rays on blood urea of rats immune to Jensen's sarcoma, *515
Pertussis: See Whooping cough
Phagocytes and Phagocytosis: See also Immunity; Leukocytes; Reticulo-Endothelial System
destruction of tubercle bacilli within phagocytes in vitro, 440
lung tissue culture, 581
Vi antigen of Bacillus typhosus and its corresponding antibody, 132
Phosphoprotein: See Blood, proteins
Photography, infra-red, of gross anatomic specimens, *67
Photosensitization: See under Light
Pigmentation, cutaneous; berlock dermatitis and allied conditions, *414
senile macular, 721
Pituitary Body, change in basophil cells in syndrome attributed to basophil adenoma, 279
effects of adrenalectomy and hypophysectomy on experimental diabetes in cat, 276
homolografting of rat pituitary grown in vitro, 123
is all colloid found in midbrain of hypophysial origin? 280
pars intermedia of hypophysis in anthropoid ape and man, 720
pathologic changes in adrenalectomized dogs, particularly in anterior lobe of hypophysis, 579
rôle of hypophysis in cancer of rat, 288
primary tumors of pituitary glands, ovaries and mammary glands in mouse, 288
tumors; subclinical adenoma of, 289
Pituitary Preparations, antigenic properties of hormones, 731
Plague, antiplague vaccine, 134
Plants, Agave lecheguilla and Nolina texana, *422
Plasmocyte, histogenesis of, 748
Plaut, A.: Botryomycosis in man, 602
Pleura, endothelioma of, 904
so-called pleural endothelioma, 737
Pneumococci, anatomic and bacteriologic findings in infections with specific types of pneumococci, including types I to XXXII, *801
antiserums, action of, 732
inadequacy of allergic inflammation as protection against infection of rabbits with virulent pneumococci, 145
toxin, 730
Pneumoconiosis, dust transport and lymph circulation in lung, 281
early changes in experimental silicosis of lungs, 127
iron pigment in anthracosis, 281
pulmonary asbestosis and carcinoma, 734
silicosis and related conditions (annual Gross lecture), 905
silicosis and tuberculosis, 125
squamous cell carcinoma of lung in asbestosis, 446
Pneumonia: See also Bronchopneumonia
anatomic and bacteriologic findings in infections with specific types of pneumococci, including types I to XXXII, *801
lipoid, of adult type (paraffinoma of lung); report of 5 cases, *470
lipoid, report of case, 608
lobar, mucin as aid in experimental production of, 883
parenteral protein in, 744
pathogenesis of pneumococcus infections in mice, 129
relations between inflammatory processes of lung and reactions of respective lymph glands, 126
Poison Ivy: See Rhus, poisoning
Poisons and Poisoning: See under names of specific substances, as Cobalt; Histamine; Nicotine; etc.
central nervous system in acute metallic poisoning, 291
Polayes, S. H.: Uterine papillary cystadenoma with origin in rests of wolffian body; report of case, 600

Poliomyelitis: See also Encephalomyelitis
experimental intranasal infection with certain neurotropic viruses; prevention by means of chemicals instilled into nostrils, 439
experimental, mechanism of immunity in, 441
experimental, olfactory bulbs in, 589
failure to infect monkeys with virus through isolated intestinal loops, 129
prevention of intravenously inoculated poliomyelitis of monkeys by intranasal instillation of picric acid, 439
protective action of certain chemicals against infection of monkeys with nasally instilled poliomyelitic virus, 890
relationship of weather conditions to poliomyelitis in state of New York, 898
respiratory versus gastro-intestinal infection in, 282
survival of virus in oral and nasal secretions of convalescents, 724
Polyarthritis: See Arthritis
Poppy seeds, encapsulation of foreign material in peritoneal cavity, 718
Porphyrin in Urine: See under Urine
Portal Vein, Obstruction: See Thrombosis, portal
Postmortems: See Autopsies
Precipitin: See also Streptococci, hemolytic
identity of precipitin and agglutinin, 895
serologic comparison of phosphoprotein of serum of laying hen and vitellin of egg yolk, 730
Pregnancy, bilateral cortical necrosis of kidney in, 434
determination of duration by erythrocyte count in capillaries of chorionic villi, 126
Prize, John Phipps memorial medal awarded to Richard E. Shope, 430
of American Association for Advancement of Science to Wendell M. Stanley, 430
offered by Eli Lilly and Company awarded to Harry Eagle, 430
Prostate, effect of avitaminosis A on, 715
topographic relations of prostate in hermaphroditism, 586
Protein: See also Amino-acids; etc.
blood plasma protein regeneration controlled by diet, 123
carbohydrate-containing proteins of hemolytic streptococcus, 440
immunologic and biochemical properties of proteins extracted from fusobacteria, anaerobic streptococci and Bacterium melanogenicum, 741
in Blood: See Blood, proteins
lesions produced by excessive ingestion of protein or irradiated food products, *95
lesions produced by parenteral injections of proteins and nephrotoxins, *245
parenteral, in pneumonias, 744
Prurigo, summer, caused by sunlight, *404
Pseudomyxoma, pseudomyxomatous epithelial growth, 736
Psittacosis, and lesions of central nervous system, 439
antigenic composition of virus of, 731
Puberty: See Adolescence
Pund, E. R.: Specific histology of granuloma inguinale, *224
Purpura, reticulo-endothelium of spleen in essential thrombopenia, 279
Pyelonephritis, inflammatory lesions in glomeruli in pyelonephritis in relation to hypertension and renal insufficiency, 432

Quinoline, production of sarcoma in mice by single subcutaneous injection of benzylamino quinoline styryl compound, 445

Rabbits, etiology of rabbit pox, 282, 437
Rabies, experimental, effect of artificial fever on, 742
Radasch, H. E.: Glomal tumors, *615
Radiations: See also Radium; Roentgen Rays; Ultraviolet Rays; and under names of various diseases
skin grafting as method of determining biologic effect of, *299
time factor in irradiation of malignant tumors, 751, *757
Radium: See also Radiations
experimental poisoning, 577
Ragins, A. B.: Pleomorphic cell sarcoma of gallbladder, 147
Raker, N.: Foreign body of long standing in biliary ducts, 747
Ramon Anatoxin: See Diphtheria, immunity; Tetanus
Recklinghausen's Disease: See Neurofibromatosis
Redish, J.: Hodgkin's disease of stomach with fatal gastric hemorrhage, 140, *844
Reed, Walter Reed medal, 122
Reichel, M.: Values of phosphatase in serum and bile of dogs with experimental biliary fistulas and in dogs with complete biliary obstruction, 739
Reiter, B. R.: Histogenesis of plasmocyte, 748
Reproduction, some effects of ovariectomy during period of declining reproductive powers in mice, 715
Respiratory Tract, diseases; common cold, 590
diseases; uncomplicated coryza of domestic fowl, 437
Reticulo-Endothelial System: See also Phagocytes and Phagocytosis
allergic lesions produced by injection of protein, 907
effect of reticulo-endothelial blockade on vaccination, 592
increase in reticulo-endothelial cells in outlying bone marrow consequent on local increase in temperature, 883
new formation of reticulum fibers, 280
reticulo-endothelium of spleen in essential thrombopenia, 279
rôle in deposition of colloidal and particulate matter in articular cavities, 579
rôle of allergy in pathogenesis of progressive thrombosis especially in regard to changes in endothelial lining of large peripheral veins, *501
Reticulosarcoma and lympho-epithelioma, 138
Reticulum: See Reticulo-Endothelial System; Tissue, connective
Rheumatic Fever, cutaneous rheumatic nodules, 124
excretion of urinary porphyrin in, 886
lesions in auriculoventricular conduction system in, 277
pericardial lesions in, 582
postmortem cultivation of tubercle bacilli in rheumatic heart disease, 893
Rhus, poisoning; hypersensitiveness to poison ivy in guinea-pigs, 897
Ribs, Ewing sarcoma in, 4 cases, 732
Rift Valley fever, mechanism of immunity to, 731
Rigdon, R. H.: Production of colitis in dog with staphylococcus toxin, *634
Robinson, H. B. G.: Ameloblastoma; survey of 379 cases from literature, *831
Histologic study of ameloblastoma, *664
Roentgen Rays: See also Radiations
effect on blood urea of rats immune to Jensen's sarcoma, *515
lesions produced by irradiation, *91
sensitivity of chromosomes to roentgen rays in relation to temperature and *p*H, 745
Therapy: See under names of diseases
Rosenblum, P.: Weltmann serum test, 143
Ruegsegger, J. M.: Anatomic and bacteriologic findings in infections with specific types of pneumococci, including types I to XXXII, *801
Rukstinat, G. J.: Allergic lesions produced by injection of protein, 907

Saccharides: See Carbohydrates
Sala, A. M.: Carcinoma of breast with condition of blood simulating chronic lymphatic leukemia, *531
Saliva, blood group ferment in, 592
group determination on dried blood spots and traces of saliva, 597
Salivary Glands: See also Submaxillary glands
disease of mice, 283
visceral lesions produced in mice by salivary gland virus of mice, 437

Salmonella, acute endocarditis produced by Bacillus paratyphosus B, *270
aertrycke; Bacterium aertrycke endotoxin, 285
pullorum, variation of, 590
Vi antigens of various Salmonella types, 441
Sandberg, M.: Rôle of adrenal cortex in production and utilization of vitamin C; its influence on structure of teeth in albino rat, *372
Sanes, S.: So-called congenital absence of left coronary artery in child, 294
Saphir, O.: Foreign body reaction of myocardium with reference to formation of giant cells and so-called myocytes, 743
Transposition of arterial trunks, 146
Sarcoma: See also Lymphosarcoma; Melanosarcoma; Reticulosarcoma
adsorption and elution of Rous sarcoma agent, 289
blood changes in mice in, 735
connection between erythroblastosis (hemocytoblastosis), myelosis and sarcoma in chickens, 290
Daels-Biltris transplantable sarcoma of guinea-pig, 735
effect of temporary stoppage of blood of rat tumors, 446
Ewing, in ribs, 4 cases, 732
Ewing's, 446
intracutaneous immunization against mouse sarcoma, 137
Jensen's, effect of roentgen rays on blood urea of rats immune to, *515
Kaposi's disease as systemic angiomatosis, 736
leukosis and sarcomatosis of chickens; unity of virus, 137
maintenance of human normal cells and tumor cells in continuous culture, 733
malignant tumors from solar irradiation, 290
morphology of sarcomas produced by 1,2,5,6-dibenzanthracene, 135
of uterus, *525
pleomorphic cell sarcoma of gallbladder, 147
production in mice by single subcutaneous injection of benzylamino quinoline styryl compound, 445
reaction of mice and mouse tumors to injections of bacterial products, 733
recurrent, of uterus, 609
skin in sarcomatosis of mice, 290
spindle cell tumors and leukemia in mice after injection of water-soluble compound of 1,2,5,6-dibenzanthracene, 901
transplantation of tumors induced by 1,2,5,6-dibenzanthracene in albino mice, 901
Scarlet fever, visceral lesions in scarlet fever and related streptococcus infections, 885
Schick Test: See Diphtheria
Schiller, W: Mesonephroma ovarii, 603
Schistosomiasis of lungs, sudden death in case of, 600
Schlezinger, N. S.: Tumor of superior thoracic inlet (tumor of superior pulmonary sulcus); clinicopathologic study, *383
Scleroderma, calcium metabolism in, 607
Sclerosis: See also Arteriosclerosis
connective tissue reaction in multiple and in diffuse sclerosis, *338
multiple, silver cells (Steiner's method) in, 278
Scorbutus: See Scurvy
Scurvy, acute, effect on guinea-pig heart, *20
Seasons, influence in development of tar tumors in mice, 288
Sebaceous Glands, transformation of nevus cells into sebaceous gland cells? 279
Secretions, Internal: See Endocrine Glands
Senility: See Old age
Septicemia, changes in spinal cord and posterior root ganglions in sepsis, 722
Septum, Interauricular: See Heart, abnormalities
Intraventricular: See under Heart
Serodiagnosis: See under names of diseases, as Syphilis; Undulant fever
Serum: See also under Blood
immune, detection of antigenic differences in mouse erythrocytes by means of, 441
Sickness: See Anaphylaxis and Allergy
Sex, Hormones: See Hormones, sex
Shear, M. J.: Studies in carcinogenesis; compounds related to benzopyrene, 746
Sheer, F.: Uterine papillary cystadenoma with origin in rests of wolffian body; report of case, 600
Shigella: See Dysentery
Shock, Anaphylactic: See Anaphylaxis and Allergy
mechanism of death following intestinal obstruction, 579
Shoulder, suprascapular branchial cyst, 889
Shwartzman Phenomenon: See Immunity
Siderosis in cats, 279
Silicosis: See Pneumoconiosis
Silicotuberculosis: See Pneumoconiosis
Simms, H. S.: Fat deposition in arteries treated in vitro with B factor, *332
Production of fat granules and of degeneration in cultures of adult tissue by agents from blood plasma, *316
Sinus, Valsalva: See Aneurysm, aortic
Skin, Diseases: See also Dermatitis
diseases; local immunization against infections of skin with staphylococci, 592
diseases; photosensitization and photodynamic diseases of man and lower animals, *399
grafting as method of determining biologic effect of radiation, *299
in sarcomatosis of mice, 290
sensitivity of different sites of skin of mouse to carcinogenic agents, 594
Slides, simple mailing container for, *849
Smallpox, vaccination; generalized vaccinia in chick embryo, 282
vaccination; intracerebral injection of vaccine virus in white mice, 727
vaccination; neurotesticular and dermal strains of vaccine virus in chorio-allantoic membrane of chick embryo, 587
vaccination; susceptibility of guinea-pig fetus to vaccinia, 128
vaccine; investigations of vaccine virus, 725
Societies, American Association of Pathologists and Bacteriologists, annual meeting, 275, 576
American Association of Public Health, meeting, 713
American Society of Clinical Pathologists, meeting, 430
Society of American Bacteriologists, new officers, 430

Society Transactions:

American Society for Experimental Pathology, 738
Buffalo Pathological Society, 294
Chicago Pathological Society, 142, 453, 606, 751, 906
Los Angeles Pathological Society, 908
New York Pathological Society, 140, 449, 600
Pathological Society of Eastern New York, 608
Pathological Society of Philadelphia, 292, 747, 905

Specimens, infra-red photography of gross anatomic specimens, *67
museum mountings at minimum cost, *536
preparation of permanent gross colonies of fungi for teaching purposes, *681
Spector, M.: Tuberculosis of appendix, 908
Spermatic cord, tumors of spermatic cord, epididymis and testicular tunics, 136
Spinal Cord: See also Meninges; Nervous System; etc.
changes in spinal cord and posterior root ganglions in sepsis, 722
dermoid cyst of, 454
effect of experimental temporary vascular occlusion on, 881
ganglioglioneuroma of, 144
Spinal Fluid: See Cerebrospinal Fluid
Spine, pathologic comparison of tuberculosis and pyogenic infection of, 454
Spirochaeta Pallida, variations in pathogenicity of spirochete of syphilis, 893

Spleen, edema of, 585
regeneration of autoplastic splenic transplants, 714
reticulo-endothelium of spleen in essential thrombopenia, 279
size of spleen and liver-spleen ratio; statistical study based on 1,000 autopsies, *33
Stains and Staining, comment on Kawamura-Yasaki fat staining method and modification in preparation of stock solution, 139
criticism of laboratory routine in modern institutions of pathology, 749
modification of Ziehl-Neelsen method of staining tubercle bacilli and tissues in paraffin or pyroxylin (celloidin) sections; comparison of this method with Cooper's technic, *265
new method for staining fat, 138
silver cells (Steiner's method) in multiple sclerosis, 278
Staphylococci, artificial opsonization of bacteria, 898
from animals with reference to toxin production, 286
local immunization against infections of skin with staphylococci, 592
production of colitis in dog with staphylococcus toxin, *634
studies in synergy, 895
toxin, and amino-acids, 133
Status Epilepticus: See Epilepsy
Stein, R. J.: Carcinoma of breast with condition of blood simulating chronic lymphatic leukemia, *531
Steinberg, B.: Transfers of living leukocytes, 743
Stewart, H. L.: Changes in liver of cat following ligation of single hepatic ducts, *641
Stillman, N. P.: Fat deposition in arteries treated in vitro with B factor, *332
Production of fat granules and of degeneration in cultures of adult tissue by agents from blood plasma, *316
Stomach: See also Gastro-Intestinal Tract
Hodgkin's disease of stomach with fatal gastric hemorrhage, 140, *844
mucin as aid in experimental production of lobar pneumonia, 883
Ulcers: See Peptic Ulcers
Stone, L.: Lesions of nervous system of rat in vitamin B deficiency, *207
Streptococci: See also Scarlet fever
anaerobic, rôle in human infections, *230
antistreptolysin titer in relation to latitude, 132
capsules about hemolytic streptococci as visualized with india ink or azo-blue, 598
effect of acute scurvy on guinea-pig heart, *20
effect of dissociation of Streptococci on their fibrinolytic and anticlotting activity, 283
hemolytic, carbohydrate-containing proteins of, 440
hemolytic, from human infections, toxigenic properties, 131
immunologic and biochemical properties of proteins extracted from fusobacteria, anaerobic streptococci and Bacterium melaninogenicum, 741
of subacute bacterial endocarditis, 591
serologic, toxigenic and biochemical reactions of hemolytic streptococci from throat of Hongkong Chinese, 893
visceral lesions in scarlet fever and related streptococcus infections, 885
Strong, L. C.: Blood of female mice (breeders) of cancer-susceptible (A) and cancer-resistant (CBA) strains, *202
Styryl 430: See under Quinoline
Subdural Space: See Meninges
Submaxillary glands, susceptibility of guinea-pig fetus to submaxillary gland virus of guinea-pigs, 128
Sudan grass, photosensitization from, *420
Sulpharsphenamine: See under Arsphenamines
Sunderland, D. A.: Effect of acute scurvy on guinea-pig heart, *20
Sunlight: See Light
Suprarenals: See Adrenals
Sweat, toxic substances in urine and sweat in typhoid fever, 284
Sweat Glands, genesis of syringoma, 444
Sympathoblastoma, intrathoracic, 136
Synovial Fluid, pathology of synovial effusions, 584
Syphilis: See also under names of organs, regions and diseases
congenital, with unusual histologic changes in myocardium, 2 cases, 450
death of 2 syphilitic infants from hemorrhagic encephalitis following treatment with sulpharsphenamine, 431
reinoculation with spirochetes of yaws, 728
serodiagnosis: influence of concentration of serum on complement fixation and precipitation, 286
specific infectiousness of syphilitic ganglions, 130
time of appearance of spirochetes of syphilis in regional lymph nodes, 727
transmission in mice, 439
variations in pathogenicity of spirochete of, 893
Syringoma, genesis of, 444

Taenia: See Tapeworm infestation
Tannenberg, J.: Experimental studies on primary changes during formation of thrombi, *307
Rôle of allergy in pathogenesis of progressive thrombosis especially in regard to changes in endothelial lining of large peripheral veins, *501
Tapeworm infestation, active immunization of albino rats with chemical fractions from cat tapeworm (Taenia taeniaformis) and its larval form (Cysticercus fasciolaris [Crassicollis]), 751
Tar Cancer: See under Cancer
relation between growth rate of tar warts in mice and autografts, 289
Teeth, ameloblastoma; survey of 379 cases from literature, *831
histologic study of ameloblastoma, *664
rôle of adrenal cortex in production and utilization of vitamin C; its influence on structure of teeth in albino rat, *372
Temperature, changes in outlying bone marrow accompanying local increase of temperature within physiologic limits, 882
increase in reticulo-endothelial cells in outlying bone marrow consequent on local increase in temperature, 883
influence on iso-agglutination, 598
sensitivity of chromosomes to roentgen rays in relation to temperature and p_H, 745
Teratoma, histogenesis of neural tissue in, 594
mediastinal, with secondary squamous cell carcinoma, 608
Testicles, gonadotropic hormone in testicular tumors, 448
preparations; histologic changes in sex organs of spayed rats induced by testosterone and estrone, 716
serologic relations between brain and testicle, 443
studies in histochemistry; vitamin C in testes in relation to anatomic and to functional changes, *363
tumors of spermatic cord, epididymis and testicular tunics, 136
Testosterone: See Testicles, preparations
Tetanus, active immunization against tetanus infection with tetanus toxoid, 897
active immunization of central nervous system against tetanus, 592
immunization against tetanus by means of Ramon's anatoxin, 443
researches on, 728
toxin in digestive tract, 286
toxoid, studies on, 730
Thannhauser, S.: Values of phosphatase in serum and bile of dogs with experimental biliary fistulas and in dogs with complete biliary obstruction, 739
Thompson, L.: Rôle of anaerobic streptococci in human infections, *230

Thorax, intrathoracic sympathoblastoma, 136
tumor of superior thoracic inlet (tumor of superior pulmonary sulcus); clinicopathologic study, *383
Throat, cytoplasmic inclusion bodies in, 590
serologic, toxigenic and biochemical reactions of hemolytic streptococci from throat of Hongkong Chinese, 893
Thrombo-angiitis obliterans, arteriographic study of collateral circulation, 433
Thrombopenia: See Purpura
Thrombophlebitis, primary idiopathic, 578
Thrombosis: See also Embolism
acute coronary, unusual findings in, 449
experimental studies on primary changes during formation of thrombi, *307
genesis of multiple encephalomalacia and of angina pectoris in foam formation, 280
portal; chronic occlusion of portal vein, 720
progressive, rôle of allergy in pathogenesis of, especially in regard to changes in endothelial lining of large peripheral veins, *501
pulmonary; sickle-cell anemia; new cause of cor pulmonale, 277
Thymoma and thymic hyperplasia in myasthenia gravis, 718
Thymus and adrenals in response of organism to injuries and intoxications, 580
extract, influence on certain endocrine organs of rat, 293
thymoma and thymic hyperplasia in myasthenia gravis, 718
Thyroid, epithelial metaplasia of thyroid gland with reference to histogenesis of squamous cell carcinoma of thyroid gland, *821
Tissue: See also Cells
Adipose: See under Fat
collagen and reticulum fibers in necrotic foci, 584
common cold, 590
connective tissue reaction in multiple and in diffuse sclerosis, *338
criticism of laboratory routine in modern institutions of pathology, 749
cultivation for prolonged periods in single flasks, 715
culture, behavior of typhus rickettsiae in, 725
culture, origin of fat and lipoids in, 717
demonstration of gold in animal tissues by method of physical development (germ method of Liesegang), 599
homiografting of rat pituitary grown in vitro, 123
hydraulic automatic tissue changer or immersion device, *392
maintenance of human normal cells and tumor cells in continuous culture, 733
modification of Ziehl-Neelsen method of staining tubercle bacilli and tissues in paraffin or pyroxylin (celloidin) sections; comparison of this method with Cooper's technic, *265
Permeability: See Osmosis and Permeability
production of fat granules and of degeneration in cultures of adult tissue by agents from blood plasma, *316
Tobacco, effects of exhaust gases from internal combustion engines and of tobacco smoke on mice, with reference to incidence of tumors of lung, 903
production of cancer in rabbits with tobacco tar, 137
Tocantins, L. M.: Technical methods for study of blood platelets; critical review with bibliography, *850
Tonsils, tuberculosis of, 909
Toxin and Antitoxin, lesions produced by bacteria and bacterial toxins, *103
Trachea, bone formation in lung and trachea, 435
immunologic studies of virus of infectious laryngotracheitis of fowls, with "developing egg technic," 895
Trauma, thymus and adrenals in response of organism to injuries and intoxications, 580
Treponema: See Spirochaeta Pallida
Tribulosis, geeldikkop, *421
Trichinosis, laboratory diagnosis in, 448
nontransmissibility in utero in rat, 742
Trichomonas foetus, antibodies for, 744
Tricuspid Valve, endocardial reaction of mitral and tricuspid valves of infants and children, 436
Trifoliosis (clover disease, etc.), *420
Trypanosomiasis, lesions of central nervous system in, 125
Tryptophan in Blood: See Blood, tryptophan
Tsutsugamushi Disease, typhus group in Malaya, 892
Tubercle Bacilli, acid-fast and alcohol-fast bacilli in urine of patients with rheumatic polyarthritis, 894
colony morphology of, 128
destruction within phagocytes in vitro, 440
dissociation, with reference to avian and human types, 890
in Blood: See Tuberculosis, bacteremia
modification of Ziehl-Neelsen method of staining tubercle bacilli and tissues in paraffin or pyroxylin (celloidin) sections; comparison of this method with Cooper's technic, *265
Tuberculin: See under Tuberculosis
Tuberculosis: See also Tubercle Bacilli; and under names of various organs and regions, as Appendix, tuberculosis; Kidneys, tuberculosis; Meningitis, tuberculous; Spine; Tonsils, tuberculosis
allergy in; nonspecific allergy, 731
and silicosis, 125
bacteremia; tuberculous bacilliemia, 723
chronic deficiency of vitamin C in tuberculosis in guinea-pig, 889
cultivation of tubercle bacilli from blood, 588
effect of heterologous bacterial products on tuberculous animals, 284
fixed antigens of tubercle bacillus, 133
irradiated milk in experimental inhalation tuberculosis in guinea-pigs, 890
pathogenesis of tuberculosis as general and systemic infection with reference to pulmonary tuberculosis, 451
postmortem cultivation of tubercle bacilli in rheumatic heart disease, 893
rôle of anoxia of tissues in reaction to tuberculin, 740
serologic specificity of tuberculous caseous matter, 443
tuberculous liquefaction, 442
variability of tuberculin reaction, 908
Tularemia, report of 3 fatal cases with autopsies, 278
Tumors: See also Adenocarcinoma; Adenoma; Arrhenoblastoma; Cancer; Epithelioma; Fibroma; Glioma; Melanoma; Sarcoma; Teratoma; and under names of organs and regions, as Brain, tumors; Breast, tumors; Kidneys, tumors; Lungs, tumors; Viscera, tumors; etc.
chemical reducing capacity and vitamin C content of transplantable tumors of rat and guinea-pig, 735
dermoid; dermoid cyst of spinal cord, 454
giant cell, genesis of, 902
glomal, *615
growth curves of malignant and normal tissues of rat, 734
in tadpole, 292
isolation of pure strains of cells from human tumors, 287
malignant, production in albino rats by ultraviolet rays, 901
mixed, of palate, 136
mixed, of salivary gland type involving finger, 447
morphology of fats in, 447
time factor in irradiation of malignant tumors, 751, *757
tumor antigens, 597
Typhoid, bacilli, immunologic study of polysaccharide of, 441
significance of Vi antigen and antibody in, 899
toxic substances in urine and sweat in typhoid fever, 284

Typhus, 130
and Proteus OX19 agglutinins, 442
attenuation of virus by bile, 894
behavior of typhus rickettsiae in tissue cultures, 725
culture strains of European and murine typhus, 437
in Malaya, 892
remarkable frequency of Proteus X19 during epidemic, 440
Vi antigen of Bacillus typhosus and its corresponding antibody, 132

Udders, inflammation; streptococci from human gastro-intestinal ulcerations and from bovine mastitis, 438
Ulcers: See Peptic Ulcers
Ultraviolet Rays: See also Light; Radiations
production of malignant tumors in albino rats by, 901
recovery of influenza virus suspended in air and its destruction by ultraviolet radiation, 587
Undulant fever, serodiagnosis of, 593
Ungar, J., Jr.: Skin grafting as method of determining biologic effect of radiation, *299
Urea in Blood: See Blood, urea
Urine, adrenal cortex, 882
bilirubin content of blood and urobilinogen content of urine in diabetes mellitus, 720
porphyrin, excretion in rheumatic fever, 886
porphyrin; hydroa vacciniforme seu aestivale with porphyrinuria, caused by sunlight, *404
toxic substances in urine and sweat in typhoid fever, 284
Urobilinogen: See under Urine
Uterus: See also Endometrium
chorio-epithelioma of, 5 cases, 453
effect of hysterectomy on ovary of Macacus rhesus, 276
Masculinus: See Müller's Duct
metaplasia of uterine epithelium in rats after administration of estrin, 577
prolonged application of follicular hormone on rabbit uterus, 714
recurrent sarcoma of, 609
sarcoma of, *525
uterine papillary cystadenoma with origin in rests of wolffian body; report of case, 600
Utricle, Prostatic: See Müller's Duct

Vaccination, Encephalitis Following: See Encephalitis
Vaccinia: See Smallpox, vaccination
Valentine, E. H.: Sternal marrow in pernicious anemia; correlation of observations at biopsy with blood picture and effects of specific treatment in megaloblastic ("liver-deficient") hyperplasia, *159
Vance, B. M.: Sudden death in case of schistosomiasis of lungs, 600
Variola: See Smallpox
Veins: See also Embolism; Thrombophlebitis; Thrombosis
hepatic, sclerosis in chronic congestive heart failure, *457
rôle of allergy in pathogenesis of progressive thrombosis especially in regard to changes in endothelial lining of large peripheral veins, *501
Verruca, relation between growth rate of tar warts in mice and autografts, 289
senile macular pigmentation, 721
Vertebra: See Spine
Viosterol, lesions produced by excessive ingestion of protein or irradiated food products, *95
Virilism, Morgagni's syndrome—internal frontal hyperostosis, virilism and obesity, 723
Viruses: See also Herpes, virus; Influenza, virus
change in rabbit fibroma virus suggesting mutation, 128
common cold, 590
effect of purified enzymes on viruses and gram-negative bacteria, 724
Viruses—Continued
epidemic in mouse colony due to virus of acute lymphocytic choriomeningitis, 590
etiology of rabbit pox, 282, 437
immunologic studies of virus of infectious laryngotracheitis of fowls, with "developing egg technic," 895
leukosis and sarcomatosis of chickens; unity of virus, 137
mass factor in immunologic studies on, 896
of infectious ectromelia, 594
propagation of virus of infectious ectromelia of mice in developing egg, 726
susceptibility of guinea-pig fetus to submaxillary gland of virus of guinea-pigs, 128
virus disease of owls, 587
visceral lesions produced in mice by salivary gland virus of mice, 437
Viscera, lesions in scarlet fever and related streptococcus infections, 885
lesions produced in mice by salivary gland virus of mice, 437
tumors, experimental production with hydrocarbons, 593
Vitamins, A; effect of avitaminosis A on prostate, 715
B, deficiency, lesions of nervous system of rat in, *207
C, chemical reducing capacity and vitamin C content of transplantable tumors of rat and guinea-pig, 735
C, chronic deficiency in tuberculosis in guinea-pig, 889
C, rôle in resistance, *543, *683
C, rôle of adrenal cortex in production and utilization of; its influence on structure of teeth in albino rat, *372
C, studies in histochemistry; vitamin C in testes in relation to anatomic and to functional changes, *363
Vitellin: See under Eggs

Wald, H.: Weight of normal adult human kidneys and its variability, *493
Wallhauser, A.: Preparation of permanent gross colonies of fungi for teaching purposes, *681
Walsh, T. E.: Experimental pulmonary edema and bronchopneumonia, 744
Warren, S.: Skin grafting as method of determining biologic effect of radiation, *299
Warts: See Verruca
Water, adrenal cortex, 882
Weather, relationship of weather conditions to poliomyelitis in state of New York, 898
Weiss, C.: Immunologic and biochemical properties of proteins extracted from fusobacteria, anaerobic streptococci and Bacterium melaninogenicum, 741
Weitzman, C. C.: Uterine papillary cystadenoma with origin in rests of wolffian body; report of case, 600
Wells, H. G.: Acute endocarditis produced by Bacillus paratyphosus B, *270
Weltmann Reaction: See Blood, coagulation
Whooping cough, experimental production in chimpanzees, 589
Witebsky, E.: Serologic demonstration of causative agent in spinal fluid in cases of meningitis, 295
Wolffian Body, uterine papillary cystadenoma with origin in rests of wolffian body; report of case, 600
Wollenweber, H. L.: Simple mailing container for slides, *849
Wright, A. W.: Monocytic leukemia; report of case, 609
Wright, R. B.: Aneurysm of sinus of Valsalva with rupture into right auricle, *679

Xanthoma, lipoid cell granuloma of fallopian tubes after uterosalpingography, 888
Xeroderma pigmentosum and skin cancer due to sunlight, *409

Yaws: See Frambesia

Zeitlin, H.: Actinomycotic abscess of brain, *58
Ganglioglioneuroma of spinal cord, 144

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CONTENTS

PAGE

THE TIME FACTOR IN THE IRRADIATION ... LIGNANT TUMORS. PERRY M. MELLICK, M.D., PH.D., ALBERT RACHEM, PH.D., CHICAGO 757

NASOPALATINE DUCT STRUCTURES AND ... AR BONY PATTERN OBSERVED IN ANTERIOR MAXIL... ION. LESTER W. BURRETT, D.D.S., M.D., NEW HA... 793

ANATOMIC AND BACTERIOLOGIC FINDIN... INFECTIONS WITH SPECIFIC TYPES OF PNEUMOCO... INCLUDING TYPES I AND XXXII. MAXWELL FINLA... D., AND JOHN W. BROWN, M.D., BOSTON, AND ... M. RUEGSEGGER, M.D., CINCINNATI 801

EPITHELIAL METAPLASIA OF THE THY... ND, WITH SPECIAL REFERENCE TO THE HISTOGE... SQUAMOUS CELL CARCINOMA OF THE THYROID GLA... H. JAFFE, M.D., CHICAGO 821

AMELOBLASTOMA: A SURVEY OF THRE... RED AND SEVENTY-NINE CASES FROM THE LITER... HAMILTON B. G. BURCHELL, M.S., D.D.S., ROCHE... Y..... 831

CASE REPORTS:

HODGKIN'S DISEASE OF THE STOMA... FATAL GASTRIC HEMORRHAGE. JULIUS REDISH, M... YORK..... 844

LABORATORY METHODS AND TECHNIC... S:

A SIMPLE MAILING CONTAINER FOR ... H. L. WOLLEN-WEBER, M.D., BALTIMORE..... 849

GENERAL REVIEW:

TECHNICAL METHODS FOR THE STU... BLOOD PLATELETS: A CRITICAL REVIEW WITH BI... Y. LEANDRO M. TOCANTINS, M.D., PHILADELPHIA 850

NOTES AND NEWS..... 880

ABSTRACTS FROM CURRENT LITERA... 881

SOCIETY TRANSACTIONS:

PATHOLOGICAL SOCIETY OF PHI... 905

CHICAGO PATHOLOGICAL SOCIET... 906

LOS ANGELES PATHOLOGICAL SO... 908

BOOK REVIEWS..... 910

BOOKS RECEIVED..... 912

GENERAL INDEX..... 913